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(71) Applicant (for all designated States except US): CURA-GEN CORPORATION [US/US]; 555 Long Wharf Drive, 11th floor, New Haven, CT 06511 (US).

(72) Inventors; and
(75) Inventors/Applicants (for US only): MEZES, Peter, S. [CA/US]; 7 Clark's Lane, Old Lyme, CT 06371 (US). RASTELLI, Luca [IT/US]; 52 Pepperbush Lane, Guilford, CT 06437 (US). HERRMANN, John, L. [US/US]; 78 Barnshed Lane, Guilford, CT 06437 (US). MAC-DOUGALL, John, R. [CA/US]; 117 Russel Street, Hamden, CT 06643 (US). ZHONG, Haihong [CN/US]; 2269 Long Hill Road, Guilford, CT 06437 (US). CASMAN, Stacie, J. [US/US]; 17 Peck Street, North Haven, CT 06473 (US). BOLDOG, Ferenc [HU/US]; 1687 Hartford Turnpike, North Haven, CT 06473 (US). SHIMKETS, Richard, A. [US/US]; 5 Indian Meadows Drive, Guilford, CT 06437 (US). GORMAN, Linda [US/US]; 392 Monticello Drive, Branford, CT 06405 (US). CRASTA, Oswald, R. [IN/US]; 4 RoseMarie Lane, Clinton, CT 06405 (US). MYSORE, Kiran, Kumar [IN/US]; 115 Pinewood Drive, Ithaca, NY 14850 (US). FOLKERTS, Otto [NL/US]; 29 Arrowhead Drive, Guilford, CT 06437 (US). MARTIN, Gregory, B. [US/US]; 30 Burdick Hill Road, Ithaca, NY 14850 (US). EISEN, Andrew [US/US]; 12412 St. James Road, Rockville, MD 20850 (US). SPADERNA, Steven, K. [US/US]; 261 Deerfield Drive, Berlin, CT 06037 (US). VERNET, Corine, A., M. [FR/US]; 1739 Foxon Road, Box L6, North Branford, CT 06471 (US). BERGH, Constance [NL/US]; 459 Orange Street, New Haven, CT 06511 (US). SPYTEK, Kimberly, A. [US/US]; 28 Court Street #1, New Haven, CT 06511 (US). DIPIPPO, Vincent, A. [US/US]; 156 North Water Street, East Haven, CN 06512 (US). ZERHUSEN, Bryan, D. [US/US]; 337 Monticello Drive, Branford, CT 06405 (US). PEYMAN, John, A. [US/US]; 336 West Rock Avenue, New Haven, CT 06515 (US). ELLERMAN, Karen [US/US]; 87 Montoya Drive, Branford, CT 06405 (US). STONE, David, J. [US/US]; 223 Whitethorn Drive, Guilford, CT 06437 (US). GROSSE, William, M. [US/US]; 15 Rice Terrace Road, Apartment C, Branford, CT 06405 (US). ALSOBROOK, John, P., II [US/US]; 60 Lake Drive, Madison, CT 06443 (US). LEPLEY, Denise, M. [US/US]; 51 Church Street, Branford, CT 06405 (US). RIEGER, Daniel, K. [US/US]; 3 Business Park Drive, Branhord, CT 06405 (US). BURGESS, Catherine, E. [US/US]; 90 Carriage Hill Drive, Weathersfield, CT 06109 (US). EDINGER, Schlomit [US/US]; 766 Edgewood Avenuet, New Haven, CT 06515 (US).

(74) Agent: ELRIFI, Ivor, R.; Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P.C, One Financial Center, Boston, MA 02111 (US).

(54) Title: PROTEINS AND NUCLEIC ACIDS ENCODING SAME

(57) Abstract: Disclosed herein are nucleic acid sequences that encode novel polypeptides. Also disclosed are polypeptides encoded by these nucleic acid sequences, and antibodies, which immunospecifically-bind to the polypeptide, as well as derivatives, variants, mutants, or fragments of the aforementioned polypeptide, polynucleotide, or antibody. The invention further discloses therapeutic, diagnostic and research methods for diagnosis, treatment, and prevention of disorders involving any one of these novel human nucleic acids and proteins.

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PROTEINS AND NUCLEIC ACIDS ENCODING SAME

FIELD OF THE INVENTION

The invention generally relates to nucleic acids and polypeptides encoded thereby.

BACKGROUND OF THE INVENTION

The invention generally relates to nucleic acids and polypeptides encoded therefrom. More specifically, the invention relates to nucleic acids encoding cytoplasmic, nuclear, membrane bound, and secreted polypeptides, as well as vectors, host cells, antibodies, and recombinant methods for producing these nucleic acids and polypeptides.

SUMMARY OF THE INVENTION

The invention is based in part upon the discovery of nucleic acid sequences encoding polypeptides. The nucleic acids and polypeptides are referred to herein as SEC1, SEC2, SEC3, SEC4, SEC5, SEC6, SEC7, SEC8, SEC9, SEC10, SEC11, and SEC12 nucleic acids and polypeptides. These nucleic acids and polypeptides, as well as derivatives, homologs, analogs and fragments thereof, will hereinafter be collectively designated as "SECX" nucleic acid or polypeptide sequences.

In one aspect, the invention provides an isolated SECX nucleic acid molecule encoding a SECX polypeptide that includes a nucleic acid sequence that has identity to the nucleic acids disclosed in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23. In some embodiments, the SECX nucleic acid molecule will hybridize under stringent conditions to a nucleic acid sequence complementary to a nucleic acid molecule that includes a protein-coding sequence of a SECX nucleic acid sequence. The invention also includes an isolated nucleic acid that encodes a SECX polypeptide, or a fragment, homolog, analog or derivative thereof. For example, the nucleic acid can encode a polypeptide at least 80% identical to a polypeptide comprising the amino acid sequences encoded by SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23. The nucleic acid can be, for example, a genomic DNA fragment or a cDNA molecule that includes the nucleic acid sequence of any of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23.

Also included in the invention is an oligonucleotide, *e.g.*, an oligonucleotide which includes at least 6 contiguous nucleotides of a SECX nucleic acid (*e.g.*, SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23) or a complement of said oligonucleotide.

Also included in the invention are substantially purified SECX polypeptides (SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24). In certain embodiments, the SECX polypeptides include an amino acid sequence that is substantially identical to the amino acid sequence of a human SECX polypeptide.

The invention is also based in part upon the discovery of nucleic acid sequences encoding novel polypeptides. The novel nucleic acids and polypeptides are referred to herein as NOVX, or NOV1, NOV2, NOV3, NOV4, NOV5, NOV6, NOV7, and NOV8 nucleic acids and polypeptides. These nucleic acids and polypeptides, as well as derivatives, homologs, analogs and fragments thereof, will hereinafter be collectively designated as "NOVX" nucleic acid or polypeptide sequences.

In one aspect, the invention provides an isolated NOVX nucleic acid molecule encoding a NOVX polypeptide that includes a nucleic acid sequence that has identity to the nucleic acids disclosed in SEQ ID NOS:25, 27, 29, 31, 33, 35, 37 and 39. In some embodiments, the NOVX nucleic acid molecule will hybridize under stringent conditions to a nucleic acid sequence complementary to a nucleic acid molecule that includes a protein-coding sequence of a NOVX nucleic acid sequence. The invention also includes an isolated nucleic acid that encodes a NOVX polypeptide, or a fragment, homolog, analog or derivative thereof. For example, the nucleic acid can encode a polypeptide at least 80% identical to a polypeptide comprising the amino acid sequences of SEQ ID NOS:26, 28, 30, 32, 34, 36, 39 and 40. The nucleic acid can be, for example, a genomic DNA fragment or a cDNA molecule that includes the nucleic acid sequence of any of SEQ ID NOS:25, 27, 29, 31, 33, 35, 37 and 39.

Also included in the invention is an oligonucleotide, *e.g.*, an oligonucleotide which includes at least 6 contiguous nucleotides of a NOVX nucleic acid (*e.g.*, SEQ ID NOS:25, 27, 29, 31, 33, 35, 37 and 39) or a complement of said oligonucleotide.

Also included in the invention are substantially purified NOVX polypeptides (SEQ ID NOS:26, 28, 30, 32, 34, 36, 39 and 40). In certain embodiments, the NOVX polypeptides include an amino acid sequence that is substantially identical to the amino acid sequence of a human NOVX polypeptide.

The invention also features antibodies that immunoselectively bind to SECX and/or NOVX polypeptides, or fragments, homologs, analogs or derivatives thereof.

In another aspect, the invention includes pharmaceutical compositions that include therapeutically- or prophylactically-effective amounts of a therapeutic and a pharmaceutically-acceptable carrier. The therapeutic can be, *e.g.*, a SECX and/or NOVX nucleic acid, a SECX and/or NOVX polypeptide, or an antibody specific for a SECX and/or NOVX polypeptide. In

5   a further aspect, the invention includes, in one or more containers, a therapeutically- or prophylactically-effective amount of this pharmaceutical composition.

In a further aspect, the invention includes a method of producing a polypeptide by culturing a cell that includes a SECX and/or NOVX nucleic acid, under conditions allowing for expression of the SECX and/or NOVX polypeptide encoded by the DNA. If desired, the

10   SECX and/or NOVX polypeptide can then be recovered.

In another aspect, the invention includes a method of detecting the presence of a SECX and/or NOVX polypeptide in a sample. In the method, a sample is contacted with a compound that selectively binds to the polypeptide under conditions allowing for formation of a complex between the polypeptide and the compound. The complex is detected, if present, thereby

15   identifying the SECX and/or NOVX polypeptide within the sample.

The invention also includes methods to identify specific cell or tissue types based on their expression of a SECX and/or NOVX.

Also included in the invention is a method of detecting the presence of a SECX and/or NOVX nucleic acid molecule in a sample by contacting the sample with a SECX and/or

20   NOVX nucleic acid probe or primer, and detecting whether the nucleic acid probe or primer bound to a SECX and/or NOVX nucleic acid molecule in the sample.

In a further aspect, the invention provides a method for modulating the activity of a SECX and/or NOVX polypeptide by contacting a cell sample that includes the SECX and/or NOVX polypeptide with a compound that binds to the SECX polypeptide in an amount

25   sufficient to modulate the activity of said polypeptide. The compound can be, *e.g.*, a small molecule, such as a nucleic acid, peptide, polypeptide, peptidomimetic, carbohydrate, lipid or other organic (carbon containing) or inorganic molecule, as further described herein.

The polynucleotides and polypeptides are used as immunogens to produce antibodies specific for the invention, and as vaccines. They are used to screen for potential agonist and

30   antagonist compounds. For example, a cDNA encoding SECX may be useful in gene therapy, and SECX may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering the diseases and disorders listed above and/or other pathologies and disorders.

The invention further includes a method for screening for a modulator of disorders or syndromes including, *e.g.*, diseases and disorders listed above and/or other pathologies and disorders and those disorders related to cell signal processing and metabolic pathway modulation. The method includes contacting a test compound with a SECX polypeptide and

5   determining if the test compound binds to said SECX polypeptide. Binding of the test compound to the SECX polypeptide indicates the test compound is a modulator of activity, or of latency or predisposition to the aforementioned disorders or syndromes.

Also within the scope of the invention is a method for screening for a modulator of activity, or of latency or predisposition to an disorders or syndromes including the diseases

10   and disorders listed above and/or other pathologies and disorders or other disorders related to cell signal processing and metabolic pathway modulation by administering a test compound to a test animal at increased risk for the aforementioned disorders or syndromes. The test animal expresses a recombinant polypeptide encoded by a SECX nucleic acid. Expression or activity of SECX polypeptide is then measured in the test animal, as is expression or activity of the

15   protein in a control animal which recombinantly-expresses SECX polypeptide and is not at increased risk for the disorder or syndrome. Next, the expression of SECX polypeptide in both the test animal and the control animal is compared. A change in the activity of SECX polypeptide in the test animal relative to the control animal indicates the test compound is a modulator of latency of the disorder or syndrome.

20   In yet another aspect, the invention includes a method for determining the presence of or predisposition to a disease associated with altered levels of a SECX polypeptide, a SECX nucleic acid, or both, in a subject (*e.g.*, a human subject). The method includes measuring the amount of the SECX polypeptide in a test sample from the subject and comparing the amount of the polypeptide in the test sample to the amount of the SECX polypeptide present in a

25   control sample. An alteration in the level of the SECX polypeptide in the test sample as compared to the control sample indicates the presence of or predisposition to a disease in the subject. Preferably, the predisposition includes diseases and disorders listed above and/or other pathologies and disorders. Also, the expression levels of the new polypeptides of the invention can be used in a method to screen for various cancers as well as to determine the

30   stage of cancers.

In a further aspect, the invention includes a method of treating or preventing a pathological condition associated with a disorder in a mammal by administering to the subject a SECX polypeptide, a SECX nucleic acid, or a SECX-specific antibody to a subject (*e.g.*, a human subject), in an amount sufficient to alleviate or prevent the pathological condition. In

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preferred embodiments, the disorder, includes the diseases and disorders listed above and/or other pathologies and disorders.

      In yet another aspect, the invention can be used in a method to identity the cellular receptors and downstream effectors of the invention by any one of a number of techniques

5   commonly employed in the art. These include but are not limited to the two-hybrid system, affinity purification, co-precipitation with antibodies or other specific-interacting molecules.

      Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can

10   be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

15   Other features and advantages of the invention will be apparent from the following detailed description and claims.

DETAILED DESCRIPTION OF THE INVENTION

      The invention is based, in part, upon the discovery of nucleic acid sequences that encode polypeptides. The nucleic acids and their encoded polypeptides are referred to

20   individually as SEC1, SEC2, SEC3, SEC4, SEC5, SEC6, SEC7, SEC8, SEC9, SEC10, SEC11, and SEC12. The nucleic acids, and their encoded polypeptides, are collectively designated herein as "SECX".

      The SECX nucleic acids of the invention include the SEC1, SEC2, SEC3, SEC4, SEC5, SEC6, SEC7, SEC8, SEC9, SEC10, SEC11, and SEC12 nucleic acids, or fragments,

25   derivatives, analogs or homologs thereof. The SECX proteins of the invention include the SEC1, SEC2, SEC3, SEC4, SEC5, SEC6, SEC7, SEC8, SEC9, SEC10, SEC11, and SEC12 polypeptides, epitopes or domains thereof, or derivatives, analogs or homologs thereof. The individual SECX nucleic acids and proteins are described below. Within the scope of this invention is a method of using these nucleic acids and peptides in the treatment or prevention

30   of a disorder related to cell signaling, adhesion, or metabolic pathway modulation.

      The SECX nucleic acids of the invention include the nucleic acids whose sequences are provided herein, or fragments thereof. The invention also includes mutant or variant nucleic acids any of whose bases may be changed from the corresponding base shown herein

while still encoding a protein that maintains its activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes

5   nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense

10   binding nucleic acids in therapeutic applications in a subject.

The SECX proteins of the invention include the proteins whose sequences are provided herein. The invention also includes mutant or variant proteins any of whose residues may be changed from the corresponding residue shown herein while still encoding a protein that maintains its activities and physiological functions, or a functional fragment thereof. The

15   invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to any of the proteins of the invention.

The SECX nucleic acids and proteins are useful in potential therapeutic applications implicated in various pathological disorders, described below. For example, a cDNA encoding the SECX protein may be useful in gene therapy, and the receptor -like protein may

20   be useful when administered to a subject in need thereof. Also within the scope of the invention is the use of a therapeutic in the manufacture of a medicament for treating or preventing disorders or syndromes including, e.g., developmental diseases; MHC I, II and III diseases (immune diseases); taste and scent detectability disorders; Burkitt's lymphoma; corticoneurogenic disease; signal transduction pathway disorders; metabolic pathway

25   disorders; retinal diseases including those involving photoreception; cell growth rate disorders; cell shape disorders; metabolic disorders; feeding disorders; control of feeding; the metabolic syndrome X; wasting disorders associated with chronic diseases; obesity; potential obesity due to over-eating or metabolic disturbances; potential disorders due to starvation (lack of appetite); diabetes; noninsulin-dependent diabetes mellitus (NIDDM1); infectious disease;

30   bacterial, fungal, protozoal and viral infections (particularly infections caused by HIV-1 or HIV-2); pain; cancer (including but not limited to neoplasm; adenocarcinoma; lymphoma; prostate cancer; uterus cancer); cancer-associated cachexia; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; Crohn's disease; multiple sclerosis; Albright hereditary ostoeodystrophy; angina

pectoris; myocardial infarction; ulcers; allergies; benign prostatic hypertrophy; and psychotic

and neurological disorders; including anxiety; schizophrenia; manic depression; delirium;

dementia; neurodegenerative disorders; Alzheimer's disease; severe mental retardation;

dentatorubro-pallidoluysian atrophy (DRPLA); hypophosphatemic rickets; autosomal

5   dominant (2) acrocallosal syndrome and dyskinesias, such as Huntington's disease or Gilles de

la Tourette syndrome; immune disorders; adrenoleukodystrophy; congenital adrenal

hyperplasia; hemophilia; hypercoagulation; idiopathic thrombocytopenic purpura;

autoimmume disease; immunodeficiencies; transplantation; Von Hippel-Lindau (VHL)

syndrome; stroke; tuberous sclerosis; hypercalceimia; cerebral palsy; epilepsy; Lesch-Nyhan

10   syndrome; ataxia-telangiectasia; leukodystrophies; behavioral disorders; addiction;

neuroprotection; cirrhosis; transplantation; systemic lupus erythematosus; emphysema;

scleroderma; ARDS; renal artery stenosis; interstitial nephritis; glomerulonephritis; polycystic

kidney disease; renal tubular acidosis; IgA nephropathy; cardiomyopathy; atherosclerosis;

congenital heart defects; aortic stenosis ; atrial septal defect (ASD); atrioventricular (A-V)

15   canal defect; ductus arteriosus; pulmonary stenosis ; subaortic stenosis; ventricular septal

defect (VSD); valve diseases; scleroderma; fertility; pancreatitis; endocrine dysfunctions;

growth and reproductive disorders; inflammatory bowel disease; diverticular disease; graft

vesus host disease; hyperthyroidism; endometriosis; hematopoietic disorders and/or other

pathologies and disorders of the like.

20   The polypeptides can be used as immunogens to produce antibodies specific for the

invention, and as vaccines. They can also be used to screen for potential agonist and

antagonist compounds. For example, a cDNA encoding a SECX protein may be useful in

gene therapy, and the SEC-like protein may be useful when administered to a subject in need

thereof. By way of nonlimiting example, the anti-SECX antibody compositions of the present

25   invention will have efficacy for treatment of patients suffering from the diseases and disorders

listed above, as well as other related or associated pathologies. The nucleic acid encoding

SECX protein, and the SECX protein of the invention, or fragments thereof, may further be

useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the

protein are to be assessed. These materials are further useful in the generation of antibodies

30   that bind immunospecifically to the substances of the invention for use in therapeutic or

diagnostic methods.

SEC1

A disclosed SEC1 (alternatively referred to as CG55688-01), comprises CYR61, a

secreted, cysteine-rich, heparin-binding protein encoded by a growth factor-inducible

immediate-early gene, which includes the 1887 base nucleotide sequence (SEQ ID NO:1)

5   shown in Table 1A. The disclosed SEC1 open reading frame ("ORF") begins at an ATG

initiation codon at nucleotides 81-83 and terminates at a ACT codon at nucleotides 1222-1224.

Putative untranslated regions upstream from the initiation codon and downstream from the

termination codon are underlined in Table 1A, and the start and stop codons are in bold letters.

Table 1A. SEC1 polynucleotide sequence (SEQ ID NO:1).

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GCGCACGGCCTGTCCGCTGCACACCAGCTTGTTGGCGTCTTCGTCGCCGCGCTCGCCCCG
GGCTACTCCTGCGCGCCACAATGAGCTCCCGCATCGCCAGGGCGCTCGCCTTAGTCGTCA
CCCTTCTCCACTTGACCAGGCTGGCGCTCTCCACCTGCCCCGCTGCCTGCCACTGCCCCC
TGGAGGCGCCCAAGTGCGCGCCGGGGAGTCGGGCTGGTCCGGGACGGCTGCGGCTGCTGTA
AGGTCTGCGCCAAGCAGCTCAACGAGGACTGCAGCAAAACGCAGCCCTGCGACCACACCA
AGGGGCTGGAATGCAACTTCGGCGCCAGCTCCACCGCTCTGAAGGGGGATCTGCAGAGCTC
AGTCAGAGGGCAGACCCTGTGAATATAACTCCAGAATCTACCAAAACGGGGAAAGTTTCC
AGCCCAACTGTAAACATCAGTGCACATGTATTGATGGCGCCGTGGGCTGCATTCCTCTGT
GTCCCCAAGAACTATCTCTCCCCAACTTGGGCTGTCCCAACCCTCGGCTGGTCAAAGTTA
CCGGGCAGTGCTGCGAGGAGTGGGTCTGTGACGAGGATAGTATCAAGGACCCCATGGAGG
ACCAGGACGGCCTCCTTGGTAAGGAGCTGGGATTCGATGCCTCCGAGGTGGAGTTGACGA
GAAACAATGAATTGATTGCAGTTGGAAAAGGCAGCTCACTGAAGCGGATCCCTGTTTTTG
GAATGGAGCCTCGCATCCGATACAACCCTTTACAAGGCCAGAAATGTATTGTTCAAACAA
CTTCATGGTCCCAGTGCTCAAAGACCTGTGGAACTGGTATCTCCACACGAGTTACCAATG
ACAACCCTGAGTGCCGCCTTGTGAAAGAAACCCGGATTTGTGAGGTGCGGCCTTGTGGAC
AGCCAGTGTACAGCAGCCTGAAAAAGGGCAAGAAATGCAGCAAGACCAAGAAATCCCCCG
AACCAGTCAGGTTTACTTACGCTGGATGTTTGAGTGTGAAGAAATACCGGCCCAAGTACT
GCGGTTCCTGCGTGGACGGCCGATGCTGCACGCCCCAGCTGACCAGGACTGTGAAGATGC
GGTTCCGCTGCGAAGATGGGGGAGACATTTTCCAAGAACGTCATGATGATCCAGTCCTGCA
AATGCAACTACAACTGCCCGCATGCCAATGAAGCAGCGTTTCCCTTCTACAGGCTGTTCA
ATGACATTCACAAATTTAGGGACTAAATGCTACCTGGGTTTCCAGGGCACACCTAGACAA
ACAAGGGAGAAGAGTGTCAGAATCAGAATCATGGAGAAAATGGGCGGGGGTGGTGTGGGT
GATGGGACTCATTGTAGAAAGGAAGCCTTGCTCATTCTTGAGGAGCATTAAGGTATTTCG
AAACTGCCAAGGGTGCTGGTGCGGATGGACACTAATGCAGCCACGATTGGAGAATACTTT
GCTTCATAGTATTGGAGCACATGTTACTGCTTCATTTTGGAGCTTGTGGAGTTGATGACT
TTCTGTTTTCTGTTTGTAAATTATTTGCTAAGCATATTTTCTCTAGGCTTTTTTCCTTTT
GGGGTTCTACAGTCGTAAAAGAGATAATAAGATTAGTTGGACAGTTTAAAGCTTTTATTC
GTCCTTTGACAAAAGTAAATGGGAGGGCATTCCATCCCTTCCTGAAGGGGGACACTCCAT
GAGTGTCTGTGAGAGGCAGCTATCTGCACTCTAAACTGCAAACAGAAATCAGGTGTTTTA
AGACTGAATGTTTTATTTATCAAAATGTAGCTTTTGGGGAGGGAGGGGAAATGTAATACT
GGAATAATTTGTAAATGATTTTAATTTTATATTCAGTGAAAAGATTTTATTTATGGAATT
AACCATTTAATAAAGAAATATTTACCT
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10

The disclosed sequence of SEC1 was derived by laboratory cloning of cDNA

fragments, by *in silico* prediction of the sequence. The cDNA fragments covering either the

full length of the DNA sequence, or part of the sequence, or both, were cloned. *In silico*

prediction was based on sequences available in CuraGen's proprietary sequence databases or

15   in the public human sequence databases, and provided either the full length DNA sequence, or

some portion thereof.

The disclosed SEC1 of this invention maps to chromosome 1p22.3. Chromosome

localization information was assigned using OMIM, the electronic northern bioinformatic tool

implemented by CuraGen Corporation, public ESTs, public literature references and/or

genomic clone homologies. This was executed to derive the chromosomal mapping of the

5   SeqCalling assemblies, Genomic clones, literature references and/or EST sequences that were

included in the invention.

The disclosed SEC1 polypeptide (SEQ ID NO:2) encoded by SEQ ID NO:1 has 381

amino acid residues, and is presented in Table 1B using the one-letter amino acid codes. The

Psort profile for SEC1 predicts that this sequence has a signal peptide and is likely to be

10   localized outside the cell with a certainty of 0.5422. In alternative embodiments, a SEC1

polypeptide is located to the endoplasmic reticulum (membrane) with a certainty of 0.1000, to

the endoplasmic reticulum (lumen) with a certainty of 0.1000, or to lysosomes with a certainty

of 0.1000. The Signal P predicts a likely cleavage site for a SEC1 peptide is between positions

24 and 25, *i.e.*, at the dash in the sequence ALS-TC.

15

Table 1B. Encoded SEC1 polypeptide sequence (SEQ ID NO:2).
MSSRIARALALVVTLLHLTRLALSTCPAACHCPLEAPKCAPGVGLVRDGCGCCKVCAKQL NEDCSKTQPCDHTKGLECNFGASSTALKGICRAQSEGRPCEYNSRIYQNGESFQPNCKHQ CTCIDGAVGCIPLCPQELSLPNLGCPNPRLVKVTGQCCEEWVCDEDSIKDPMEDQDGLLG KELGFDASEVELTRNNELIAVGKGSSLKRIPVFGMEPRIRYNPLQGQKCIVQTTSWSQCS KTCGTGISTRVTNDNPECRLVKETRICEVRPCGQPVYSSLKKGKKCSKTKKSPEPVRFTY AGCLSVKKYRPKYCGSCVDGRCCTPQLTRTVKMRFRCEDGETFSKNVMMIQSCKCNYNCP HANEAAFPFYRLFNDIHKFRD

Public and proprietary sequence databases were searched for protein sequences with

homology to SEC1 using BLASTP software. In all BLAST alignments herein, the "E-value"

or "Expect" value is a numeric indication of the probability that the aligned sequences could

20   have achieved their similarity to the BLAST query sequence by chance alone, within the

database that was searched. The Expect value (E) is a parameter that describes the number of

hits one can "expect" to see just by chance when searching a database of a particular size. It

decreases exponentially with the Score (S) that is assigned to a match between two sequences

of a database of comparable complexity. Essentially, the E value describes the random

25   background noise that exists for matches between sequences.

The E value is used as a convenient way to create a significance threshold for reporting

results. The default value used for blasting is typically set to 0.0001. In BLAST 2.0, the E

value is also used instead of the P value (probability) to report the significance of matches.

For example, an E value of one assigned to a hit can be interpreted as meaning that in a

30   database of the current size one might expect to see one match with a similar score simply by

chance. An E value of zero means that one would not expect to see any matches with a similar score simply by chance. See, e.g., http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/. Occasionally, a string of X's or N's will result from a BLAST search. This is a result of automatic filtering of the query for low-complexity sequence that is performed to prevent

5   artifactual hits. The filter substitutes any low-complexity sequence that it finds with the letter "N" in nucleotide sequence (e.g., "NNNNNNNNN") or the letter "X" in protein sequences (e.g., "XXX"). Low-complexity regions can result in high scores that reflect compositional bias rather than significant position-by-position alignment (Wootton and Federhen, *Methods Enzymol* 266:554-571, 1996).

10   A BLAST analysis of SEC1 was run against the proprietary PatP GENESEQ Protein Patent database. The amino acid sequence of SEC1 has high homology to other proteins as shown in Table 1C.

Table 1C. BLASTX results from PatP database for SEC1		
Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum Probability P(N)
patp:AAB43987 Human cancer associated protein	2107	2.1e-219
patp:AAW35957 Human monocyte mature differentiation factor	2107	6.6e-218
patp:AAB90773 Human shear stress-response protein	2107	6.6e-218
patp:AAW35730 Human cysteine rich protein 61 (Cyr61)	2098	5.9e-217
patp:AAE05921 Human cysteine-rich protein (Cyr61)	2098	5.9e-217

15   In a search of public sequence databases, it was found, for example, that the amino acid sequence of the protein of the invention was found to have 381 of 381 amino acid residues (100%) identical to the 381 amino acid CYR61 protein from Homo sapiens (NM_001554 protein, E=1.3e-93).

SEC1 also has homology to the other proteins shown in the BLASTP data in Table 1D.

20

Table 1D. SEC1 BLASTP results					
Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi|4504613|ref|NP_001545.1 ⊥ (NM_001554)	cysteine-rich, angiogenic inducer, 61; cysteine-rich heparin-binding protein 61; cysteine-rich, anigogenic inducer, 61 [Homo sapiens]	381	381/381 (100)	381/381 (100)	0.0

gi|13638596|r ef|XP_001831. 2| (XM_001831)	cysteine-rich, angiogenic inducer, 61 [Homo sapiens]	381	379/381 (99)	380/381 (99)	0.0
gi|2791898|em b|CAA72167.1| (Y11307)	CYR61 protein [Homo sapiens]	381	378/381 (99)	380/381 (99)	0.0
gi|12584866|g b|AAG59863.1| AF307860_1 (AF307860)	CYR61 protein [Homo sapiens]	381	378/381 (99)	379/381 (99)	0.0
gi|6753594|re f|NP_034646.1 | (NM_010516)	cysteine rich protein 61; insulin-like growth factor binding protein 10; intermediate early gene [Mus musculus]	379	344/383 (89)	352/383 (91)	e-166

A sequence alignment is given in Table 1E, with the SEC1 protein being shown on line 1 in a ClustalW analysis comparing the protein of the invention with the related protein sequences shown in Table 1D.

5

Table 1E. ClustalW

```
1) SEC1 (SEQ ID NO:2)
2) gi|4504613|   (SEQ ID NO:41)
3) gi|13638596|  (SEQ ID NO:42)
4) gi|12584866|  (SEQ ID NO:43)
5) gi|6753594|   (SEQ ID NO:44)
6) gi|6753594|   (SEQ ID NO:45)

                      10        20        30        40        50
                ....|....|....|....|....|....|....|....|....|....|
SEC1            MSSRIARALALVVTLLHLTRLALSTCPAACHCPLEAPKCAPGVGLVRDGC
gi|4504613|     MSSRIARALALVVTLLHLTRLALSTCPAACHCPLEAPKCAPGVGLVRDGC
gi|13638596|    MSSRIARALALVVTLLHLTRLALSTCPAACHCPLEAPKCAPGVGLVRDGC
gi|2791898|     MSSRIARALALVVTLLHLTRLALSTCPAACHCPLEAPKCAPGVGLVRDGC
gi|12584866|    MSSRIARALALVVTLLHLTRLALSTCPAACHCPLEAPKCAPGVGLVRDGC
gi|6753594|     MSSSTFRTLAVAVTLLHLTRLALSTCPAACHCPLEAPKCAPGVGLVRDGC

                      60        70        80        90       100
                ....|....|....|....|....|....|....|....|....|....|
SEC1            GCCKVCAKQLNEDCSKTQPCDHTKGLECNFGASSTALKGICRAQSEGRPC
gi|4504613|     GCCKVCAKQLNEDCSKTQPCDHTKGLECNFGASSTALKGICRAQSEGRPC
gi|13638596|    GCCKVCAKQLNEDCSKTQPCDHTKGLECNFGASSTALKGICRAQSEGRPC
gi|2791898|     GCCKVCAKQLNEDCSKTQPCDHTKGLECNFGASSTALKGICRAQSEGRPC
gi|12584866|    GCCKVCAKQLNEDCSKTQPCDHTKGLECNFGASSTALKGICRAQSEGRPC
gi|6753594|     GCCKVCAKQLNEDCSKTQPCDHTKGLECNFGASSTALKGICRAQSEGRPC

                     110       120       130       140       150
                ....|....|....|....|....|....|....|....|....|....|
SEC1            EYNSRIYQNGESFQPNCKHQCTCIDGAVGCIPLCPQELSLPNLGCPNPRL
gi|4504613|     EYNSRIYQNGESFQPNCKHQCTCIDGAVGCIPLCPQELSLPNLGCPNPRL
gi|13638596|    EYNSRIYQNGESFQPNCKHQCTCIDGAVGCIPLCPQELSLPNLGCPNPRL
gi|2791898|     EYNSRIYQNGESFQPNCKHQCTCIDGAVGCIPLCPQELSLPNLGCPNPRL
gi|12584866|    EYNSRIYQNGESFQPNCKHQCTCIDGAVGCIPLCPQELSLPNLGCPNPRL
gi|6753594|     EYNSRIYQNGESFQPNCKHQCTCIDGAVGCIPLCPQELSLPNLGCPNPRL

                     160       170       180       190       200
                ....|....|....|....|....|....|....|....|....|....|
SEC1            VKVTGQCCEEWVCDEDSIKDPMEDQDGLLGKELGFDASEVELTRNNELIA
gi|4504613|     VKVTGQCCEEWVCDEDSIKDPMEDQDGLLGKELGFDASEVELTRNNELIA
gi|13638596|    VKVTGQCCEEWVCDEDSIKDPMEDQDGLLGKELGFDASEVELTRNNELIA
gi|2791898|     VKVTGQCCEEWVCDEDSIKDPMEDQDGLLGKELGFDASEVELTRNNELIA
```

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gi|12584866|  VKVTGQCCEEWVCDEDSIKDPMEDQDGLLGKELGFDASEVELTRNNELIA
gi|6753594|   VKVSGQCCEEWVCDEDSIKDSLDDQDDLLG----LDASEVELTRNNELIA

                      210       220       230       240       250
              ....|....|....|....|....|....|....|....|....|....|
SEC1          VGKGSSLKRIPVFGMEPRIRYNPLQ--GQKCIVQTTSWSQCSKTCGTGIS
gi|4504613|   VGKGSSLKRIPVFGMEPRIRYNPLQ--GQKCIVQTTSWSQCSKTCGTGIS
gi|13638596|  VGKGSSLKRLPVFGMEPRILYNPLQ--GQKCIVQTTSWSQCSKTCGTGIS
gi|2791898|   VGKGSSLKRLPVFGMEPRILYNPLQ--GQKCIVQTTSWSQCSKTCGTGIS
gi|12584866|  VGKGSSLKRLPVFGMEPRILYNPLQ--GQKCIVQTTSWSQCSKTCGTGIS
gi|6753594|   IGKGSSLKRLPVFGTEPRVLSNPLHAHGQKCIVQTTSWSQCSKSCGTGIS

                      260       270       280       290       300
              ....|....|....|....|....|....|....|....|....|....|
SEC1          TRVTNDNPECRLVKETRICEVRPCGQPVYSSLKKGKKCSKTKKSPEPVRF
gi|4504613|   TRVTNDNPECRLVKETRICEVRPCGQPVYSSLKKGKKCSKTKKSPEPVRF
gi|13638596|  TRVTNDNPECRLVKETRICEVRPCGQPVYSSLKKGKKCSKTKKSPEPVRF
gi|2791898|   TRVTNDNPECRLVKETRICEVRPCGQPVYSSLKKGKKCSKTKKSPEPVRF
gi|12584866|  TRVTNDNPECRLVKETRICEVRPCGQPVYSSLKKGKKCSKTKKSPEPVRF
gi|6753594|   TRVTNDNPECRLVKETRICEVRPCGQPVYSSLKKGKKCSKTKKSPEPVRF

                      310       320       330       340       350
              ....|....|....|....|....|....|....|....|....|....|
SEC1          TYAGCLSVKKYRPKYCGSCVDGRCCTPQLTRTVKMRFRCEDGETFSKNVM
gi|4504613|   TYAGCLSVKKYRPKYCGSCVDGRCCTPQLTRTVKMRFRCEDGETFSKNVM
gi|13638596|  TYAGCLSVKKYRPKYCGSCVDGRCCTPQLTRTVKMRFRCEDGETFSKNVM
gi|2791898|   TYAGCLSVKKYRPKYCGSCVDGRCCTPQLTRTVKMRFRCEDGETFSKNVM
gi|12584866|  TYAGCLSVKKYRPKYCGSCVDGRCCTPQLTRTVKMRFRCEDGETFSKNVM
gi|6753594|   TYAGCSSVKKYRPKYCGSCVDGRCCTPLQTRTVKMRFRCEDGEMFSKNVM

                      360       370       380
              ....|....|....|....|....|....|...
SEC1          MIQSCKCNYNCPHANEAAFPFYRLFNDIHKFRD
gi|4504613|   MIQSCKCNYNCPHANEAAFPFYRLFNDIHKFRD
gi|13638596|  MIQSCKCNYNCPHANEAAFPFYRLFNDIHKFRD
gi|2791898|   MIQSCKCNYNCPHANEAAFPFYRLFNDIHKFRD
gi|12584866|  MIQSCKCNYNCPHANEAAFPLYRLFNDIHKFRD
gi|6753594|   MIQSCKCNYNCPHPNEASFRLYSLFNDIHKFRD
```

DOMAIN ANALYSIS

The presence of identifiable domains in SEC1, as well as all other SECX proteins, was determined by searches using software algorithms such as PROSITE, DOMAIN, Blocks, Pfam, ProDomain, and Prints, and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (http:www.ebi.ac.uk/ interpro). DOMAIN results for SEC1 as disclosed in Table 1F, were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST analyses. This BLAST analysis software samples domains found in the Smart and Pfam collections. For Table 1F and all successive DOMAIN sequence alignments, fully conserved single residues are indicated by the sign (|) and "strong" semi-conserved residues are indicated by the sign (+). The "strong" group of conserved amino acid residues may be any one of the following groups of amino acids: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

Table 1F lists the domain description from DOMAIN analysis results against SEC1. This indicates that the SEC1 sequence has properties similar to those of other proteins known to contain this domain.

Table 1F. Domain Analysis of SEC1
gnl|Pfam|pfam00007, Cys_knot, Cystine-knot domain. The family comprises glycoprotein hormones and the C-terminal domain of various extracellular proteins. **CD-Length = 109 residues, 89.0% aligned** **Score = 51.6 bits (122), Expect = 8e-08**

```
SEC 1:   293   PEPVRFTYAGCLSVKKYRPKYC-GSCVD-------------GRCCTPQLTRTVKMRFRC   337
                     +   GC S K      C G C              CC P +T   K+   C
Sbjct:    12   NVTISVEKEGCTSCKTVNTTICAGYCYTKDPVYKDGRSLLIQCVCCYPDVTYETKVLPGC   71

SEC 1:   338   EDGETFSKNVMMIQSCKCNYNCPHANEAAFPFYRLFND   375
                     G   +K   +   SC C    C   N          +
Sbjct:    72   PPGVDPTKTYPVALSCHCG-KCNTDNTDCTRLSLQPDS   108   (SEQ ID NO: 299)
```

5   CYR61 is a secreted, cysteine-rich, heparin-binding protein encoded by a growth factor-inducible immediate-early gene. Acting as an extracellular matrix-associated signaling molecule, CYR61 is an angiogenic inducer that promotes the adhesion of endothelial cells through interaction with integrins and augments growth factor-induced DNA synthesis in the
10   same cell type (see, Expression of cyr61, a growth factor-inducible immediate-early gene. O'Brien TP, et al., Mol Cell Biol. 1990 Jul;10(7):3569-77, incorporated by reference). CYR61 stimulates directed migration of human microvascular endothelial cells in culture through the alpha(V)beta(3)-dependent pathway and induces neovascularization of the surrounding tissues. Modulation of CYR61 provides for methods of treating disorders of angiogenesis. For
15   example, specific anti-CYR61 antibodies are useful to block both the chemotactic and angiogenic activities of CYR61, and provide a method for detection of the CYR61 polypeptide in disease states, for example, in the detection and treatment of tumorigenesis. Upregulation or expression of CYR61 is useful to promote neovascularization, for example, to promote wound healing or in transplant grafts.
20   Through a proprietary technology designed to identify transcripts of all expressed genes, a   cDNA designated CG55688-01 was isolated. In vitro, the recombinant protein was systematically tested in a number of endothelial cell assays and found to induce a critical process involved in the angiogenic cascade. First, CG55688-01 did not inhibit VEGF/bFGF-mediated proliferation or by itself induce endothelial proliferation. However, CG55688-01
25   significantly enhanced endothelial cell migration through enhanced matrix adhesion. These findings reveal a   function for CG55688-01 as playing a role in a key process of angiogenesis.

The molecule is a target for small molecules or antibodies as a cancer therapeutic or other diseases, such as psoriasis, for example, where inflammation is enhanced by increased vascularization. Alternatively, CG55688 is a therapeutic useful in wound healing, stroke or cardiovascular diseases.

5

Expression Purification and Biochemical Characterization of Recombinant SEC1

The CG55688-01 nucleic acid sequence disclosed as SEQ ID NO 1 was cloned into the pCEP4 vector (Invitrogen, Carlsbad, CA) and was transfected into HEK293T cells using Lipofectamine Plus reagent according to manufacturer's instructions (Life Technologies Inc.,

10   Rockville, MD). The cell pellet and supernatant were harvested 72 h after transfection and examined for protein expression by Western blot analysis with an anti-V5 monoclonal antibody. After initial confirmation of expression, large-scale transfections were carried out in 150-cm^2 petri dishes using Lipofectamine Plus reagent. The conditioned medium was collected from the transfected cells after 72 h, pooled and loaded onto a Ni^{2+} affinity column

15   according to manufacturer's instructions (Qiagen, Valencia, CA). The column was washed with 10 column volumes of PBS, pH 7.4 containing 500 mM NaCl. Non-specifically bound proteins were washed with above buffer containing 5 mM imidazole. The bound proteins were eluted with PBS, pH 7.4 containing 500 mM imidzaole. Peak fractions from the 500 mM imidazole peak were pooled and dialyzed overnight in PBS, pH 7.4. The protein was

20   further purified by a second round of purification over a Ni^{2+} affinity column and dialyzed against PBS, pH 7.4. The concentration of protein was measured by the Bradford reagent (Bio-Rad, Hercules, CA). Protein purity was assessed by Coomassie Brilliant Blue staining after analysis by SDS-PAGE on a 4 -15% Tris/glycine gradient gel. Purified CG55688-01 was also transferred to PVDF membrane and subsequently processed for 15 cycles of N-

25   terminal sequence analysis. In addition, the purified protein was tested for endotoxin using the Gel clot method (Cape Cod Associates, CapeCod, MA) and found to have <10 EU/mg of protein. Subsequently, the purified protein was used in all *in vitro* angiogenesis assays.

Endothelial Cell BrdU Incorporation Assay

30   Purified CG55688-01 was tested for its ability to induce or inhibit the proliferation of endothelial cells using a BrdU incorporation assay. Proliferative activity is measured by treatment of serum-starved cultured cells with a given agent and measurement of BrdU incorporation during DNA synthesis. The proliferative and antiproliferative effect of CG55688-01 was assessed using HUVEC and HMVEC-d. Cells were seeded into wells at

4x10^4 cells/well, were synchronized by serum starvation overnight in minimal medium (0.5% FBS) and stimulated with VEGF and bFGF at 10 ng/ml in the presence of 1% FBS. Different concentrations of purified protein were added along with VEGF, and incubated for 18 hours. BrdU was then added, a 4-hour incubation allowed, and ELISA was performed. The cells

5   were plated in 96-well flat bottom plates pre-coated with Attachment Factor (Cascade Biologics, Portland, OR) at 3 x10^4 cells/well in 100 µl of Medium 200 (Cascade Biologics, Portland, OR) containing 0.5 % FBS. After 24 hours of starvation at 37°C, the cells were washed twice with serum-free medium, and then fed with fresh medium containing 1% FBS with VEGF$_{165}$ and bFGF (10 ng/ml) (R & D Systems, Minneapolis, MN) with and without

10   CG55688-01 protein. The cells were pulsed with BrdU for 4 hours before harvest. The BrdU assay was performed according to the manufacturer's specification (Roche Molecular Biochemicals, Indianapolis, IN).

      Migration assay

15   To determine the ability of recombinant CG55688-01 to block migration of HUVEC and HMVEC-d towards VEGF$_{165}$, 24-well transwell (BD Biosciences, Bedford, MA) migration chambers having an 8 µm pore size were used. The transwells were coated with 10 µg/ml of Type I collagen (BD Biosciences, Bedford, MA) from rat tail for 1 h at 37 °C. After washing with PBS, the wells were seeded with HUVEC suspended at 2 x10^7 cells/ml in

20   Medium 200 containing 1% BSA (Sigma Chemicals, St Louis, MO). The lower chambers (600 µl) were filled with Medium 200 containing 1% FBS supplemented with 10 ng/ml of recombinant VEGF$_{165}$. The upper chamber was seeded with 4 x10^4 cells/well in 200 µl containing different concentrations of CG55688-01. Cells were allowed to migrate for 4 h at 37 °C. Following incubation, cells on the upper surface of the membrane (non-migrated

25   cells) were scraped with a cotton swab. Cells on the lower side of the membrane (migrated cells) were stained with 0.2 % Crystal Violet dye (Fisher Scientific, Springfield, NJ) in 70 % ethanol for 30 min. The cells were then destained in PBS, pH 7.4 and the membrane was left to air dry at room temperature. The number of cells that had migrated was counted using a Zeiss Axiovert 100 inverted microscope at three independent areas and the mean number of

30   migrated cells was calculated. RGD control peptide (Life Technologies Inc., Rockville, MD) was used as a positive control for each experiment. The number of cells migrating in the presence or absence of CG55688-01 was counted in three independent fields. The number of cells migrated in the presence of 1 % FBS with VEGF (10 ng/ml) was considered as

maximum migration and the percentage of inhibition was calculated. The results demonstrate that CG55688-01 specifically affects migration of large and small vessel endothelial cells.

Endothelial cell adhesion assay

Untreated 96-well flat bottom tissue culture plates (Fisher Scientific, Springfield, NJ) were used in the cell adhesion assay. The plates were coated with 10 μg/ml of different extracellular matrix (ECM) proteins (Type I collagen, Type IV collagen, fibronectin, vitronectin, laminin and Matrigel) overnight at 4 °C. The remaining protein binding sites were blocked with 1 % BSA in PBS, pH 7.4 for 2 h at 37 °C. HUVEC were grown to subconfluence (70-80%) in Medium 200. The cells were labeled with Calcein-AM fluorophore (Molecular Probes, Eugene, OR) as described by the manufacturer. The cells were trypsinized, washed and resuspended at 1.5×10^5 cells/ml in serum-free medium containing 1% BSA. The cells were then mixed with different concentrations of CG55688-01 or the absence of CG55688-01 in 100-μl volumes containing 2×10^4 cells/treatment for 15 min at room temperature. After incubation, the cell suspension was then added to each well and the plates were incubated at 37 °C for 45 min in 5% CO_2. At the end of the incubation period, unattached cells were removed by washing 3 times with serum-free medium, and attached cells were counted using a Cytofluor 4000 flurometer (PE Applied Biosystems, Foster City, CA). The number of attached cells was represented as percentage of endothelial cell adhesion. Typically, greater than 90 % of cells were labeled with Calcein AM fluorescence dye. In the presence of different concentrations of CG55688-01, there was a dose-enhancement of cell adhesion to ECM-coated plates indicating that CG55688-01 enhances endothelial cell adhesion to different ECM proteins, with the most pronounced effect observed on fibronectin and vitronectin.

The disclosed SEC1 comprises several domains, such as the following InterPro Domains: cystine-knot domain, C-terminal cystine knot-like domain (CTCK), von Willebrand factor (vWF) type C domain, and Insulin growth factor-binding protein homologues. The homologies shown in the tables, and disclosed above indicates that the SEC1 sequences of the invention have properties similar to those of other proteins known to contain this/these domain(s) as well as properties similar to the properties of these domains.

The CYR61 disclosed herein as SEC1 is expressed in at least the following tissues: apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye,

frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, those that express MHC I, II, and III, nervous tissues, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine,

5   smooth muscle (coronary artery in aortia) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, public EST sources, literature sources, and/or RACE sources. Further expression data for SEC1 is provided in Example 2.

10   The nucleic acids and proteins of SEC1 are useful in potential therapeutic applications implicated in various pathological disorders described above. The SEC1 nucleic acid encoding the CYR61-like protein of the invention, or fragments thereof, is useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein is assessed. These materials are further useful in the generation of antibodies that bind immunospecifically

15   to the substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-SECX Antibodies" section below. The disclosed SEC1 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, for example, a contemplated SEC1 epitope comprises from

20   about amino acids 60 to about 80. In another embodiment, for example, a SEC1 epitope comprises from about amino acids 85 to about 130. In further embodiments, for example, a SEC1 epitope comprises from about 131 to about 205, and from about 207 to about 381.

SEC2

25   The disclosed SEC2 (alternatively referred to herein as CG54933-01) includes the 2114 nucleotide sequence (SEQ ID NO:3) shown in Table 2A. A SEC2 ORF begins with a Kozak consensus ATG initiation codon at nucleotides 100-102 and ends with a TGA codon at nucleotides 1984-1986. Putative untranslated regions upstream from the initiation codon and downstream from the termination codon are underlined in Table 2A, and the start and stop

30   codons are in bold letters.

Table 2A. SEC2 Nucleotide Sequence (SEQ ID NO:3)
AGGAATTCCGGTGGCCGGCCACTCCCGTCTGCTGTGACGCGCGGACAGAGAGCTACCGGT GGACCCACGGTGCCTCCCTCCCTGGGATCTACACAGACCATGGCCTTGCAACGGCTCGAC

```
CCCTGTTGGTCCTGTGGGGACCGCCCTGGCAGCCTCCTGTTCCTGCTCTTCAGCCTCGGA
TGGGTGCATCCCGCGAGGACCCTGGCTGGAGAGACAGGGACGGAGTCTGCCCCCCTGGGG
GGAGTCCTGACAACCCCCCATAACATTTCCAGCCTCTCCCCTCGCCAACTCCTTGGCTTC
CCGTGTGCGGAGGTGTCCGGCCTGAGCACGGAGCGTGTCCGGGAGCTGGCTGTGGCCTTG
GCACAGAAGAATGTCAAGCTCTCAACAGAGCAGCTGCGCTGTCTGGCTCACCGGCTCTCT
GAGCCCCCCGAGGACCTGGACGCCCTCCCATTGGACCTGCTGCTATTCCTCAACCCAGAT
GCGTTCTCGGGGCCCCAGGCCTGCACCCGTTTCTTCTCCCGCATCACGAAGGCCAATGTG
GACCTGCTCCCGAGGGGGGGCTCCCGAGCGACAGCGGCTGCTGCCTGCGGCTCTGGCCTGC
TGGGGTGTGCGGGGGTCTCTGCTGAGCGAGGCTGATGTGCGGGCTCTGGGAGGCCTGGCT
TGCGACCTGCCTGGGCGCTTTGTGGCCGAGTCGGCCGAAGTGCTGCTACCCCGGCTGGTG
AGCTGCCCGGGACCCCTGGACCAGGACCAGCAGGAGGCAGCCAGGGCGGCTCTGCAGGGC
GGGGGGACCCCCCTACGGCCCCCCGTCGACATGGTCTGTCTCCACGATGGACGCTCTGCGG
GGCCTGCTGCCCGTGCTGGGGCCAGCCCATCATCCGCAGCATCCCGCAGGGCATCGTGGCC
GCGTGGCGGCAACGCTCCTCTCGGGACCCATCCTGGCGGCAGCCTGAACGGACCATCCTC
CGGCCGCGGTTCCGGCGGGAAGTGGAGAAGACAGCCTGTCCTTCAGGCAAGAAGGCCCGC
GAGATAGACGAGAGCCTCATCTTCTACAAGAAGTGGGAGCTGGAAGCCTGCGTGGATGCG
GCCCTGCTGGCCACCCAGATGGACCGCGTGAACGCCATCCCCTTCACCTACGAGCAGCTG
GACGTCCTAAAGCATAAACTGGATGAGCTCTACCCACAAGGTTACCCCGAGTCTGTGATC
CAGCACCTGGGCTACCTCTTCCTCAAGATGAGCCCTGAGGACATTCGCAAGTGGAATGTG
ACGTCCCTGGAGACCCTGAAGGCTTTGCTTGAAGTCGACAAAGGGCACGAAATGAGTCCT
CAGGCTCCTCGGCGGCCCCTCCCACAGGTGGCCACCCTGATCGACCGCTTTGTGAAGGGA
AGGGGCCAGCTAGACAAAGACACCCTAGACACCCTGACCGCCTTCTACCCTGGGTACCTG
TGCTCCCTCAGCCCCGAGGAGCTGAGCTCCGTGCCCCCCAGCAGCATCTGGGCGGTCAGG
CCCCAGGACCTGGACACGTGTGACCCAAGGCAGCTGGACGTCCTCTATCCCAAGGCCCGC
CTTGCTTTCCAGAACATGAACGGGTCCGAATACTTCGTGAAGATCCAGTCCTTCCTGGGT
GGGGCCCCCACGGAGGATTTGAAGGCGCTCAGTCAGCAGAATGTGAGCATGGACTTGGCC
ACGTTCATGAAGCTGCGGACGGATGCGGTGCTGCCGTTGACTGTGGCTGAGGTGCAGAAA
CTTCTGGGACCCCACGTGGAGGGCCTGAAGGCGGAGGAGCGGCACCGCCCGGTGCGGGAC
TGGATCCTACGGCAGCGGCAGGACGACCTGGACACGCTGGGGCTGGGGCTACAGGGCGGC
ATCCCCAACGGCTACCTGGTCCTAGACCTCAGCGTGCAAGAGACCCTCTCGGGGACGCCC
TGCCTCCTAGGACCTGGACCTGTTCTCACCGTCCTGGCACTGCTCCTAGCCTCCACCCTG
GCCTGAGGGCCCCACTCCCTTGCTGGCCCCAGCCCTGCTGGGGATCCCCGCCTGGCCAGG
AGCAGGCACGGGTGATCCCCGTTCCACCCCAAGAGAACTCGCGCTCAGTAAACGGGAACA
TGCCCCCTGCAGAC
```

The SEC2 polypeptide (SEQ ID NO:4) encoded by SEQ ID NO:3 is 628 amino acids in length and is presented using the one-letter amino acid code in Table 2B. The Psort profile for SEC2 predicts that this sequence has a signal peptide and is likely to be localized at the

5   plasma membrane with a certainty of 0.9190. In alternative embodiments, a SEC2 polypeptide is located to lysosomes with a certainty of 0.3000, to the endoplasmic reticulum (membrane) with a certainty of 0.1000, or to the nucleus with a certainty of 0.1800. The Signal P predicts a likely cleavage site for a SEC2 peptide is between positions 34 and 35, *i.e.*, at the dash in the sequence ART-LA.

10

Table 2B. SEC2 protein sequence (SEQ ID NO:4)
MALQRLDPCWSCGDRPGSLLFLLFSLGWVHPARTLAGETGTESAPLGGVLTTPHNISSLS PRQLLGFPCAEVSGLSTERVRELAVALAQKNVKLSTEQLRCLAHRLSEPPEDLDALPLDL LLFLNPDAFSGPQACTRFFSRITKANVDLLPRGAPERQRLLPAALACWGVRGSLLSEADV RALGGLACDLPGRFVAESAEVLLPRLVSCPGPLDQDQQEAARAALQGGGPPYGPPSTWSV STMDALRGLLPVLGQPIIRSIPQGIVAAWRQRSSRDPSWRQPERTILRPRFRREVEKTAC PSGKKAREIDESLIFYKKWELEACVDAALLATQMDRVNAIPFTYEQLDVLKHKLDELYPQ GYPESVIQHLGYLFLKMSPEDIRKWNVTSLETLKALLEVDKGHEMSPQAPRRPLPQVATL IDRFVKGRGQLDKDTLDTLTAFYPGYLCSLSPEELSSVPPSSIWAVRPQDLDTCDPRQLD VLYPKARLAFQNMNGSEYFVKIQSFLGGAPTEDLKALSQQNVSMDLATFMKLRTDAVLPL TVAEVQKLLGPHVEGLKAEERHRPVRDWILRQRQDDLDTLGLGLQGGIPNGYLVLDLSVQ ETLSGTPCLLGPGPVLTVLALLLASTLA

A BLAST analysis of SEC2 was run against the proprietary PatP GENESEQ Protein

Patent database. It was found, for example, that the amino acid sequence of SEC2 had high

homology to other proteins as shown in Table 2C.

5

Table 2C. BLASTX results from PatP database for SEC2		
Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum Probability P(N)
patp:AAW26674 Human CAK1 antigen (mesothelin)	3261	0.0
patp:AAR53992 Megakaryoctye potentiator	3047	1.8e-317
patp:AAB08544 Mesothelin related antigen (MRA)	1538	1.3e-157
patp:AAB08547 Soluble mesothelin related (SMR)	1538	1.3e-157
patp:AAB08543 Mesothelin related antigen (MRA)	1522	6.5e-156

In a search of public sequence databases, it was found, for example, that the amino

acid sequence of the SEC2 protein of the present invention was found to have 614 of 614

amino acid residues (100%) identical to the 628 amino acid NM-013404 protein from Homo

10   sapiens. SEC2 also has homology to the other proteins shown in the BLASTP data in Table

2D.

Table 2D. SEC2 BLASTP results					
Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positive (%)	Expect
gi|7108356|ref|NP_037536.1| (NM_013404)	mesothelin isoform 2 precursor; mesothelin isoform 1 precursor; megakaryocyte potentiating factor [Homo sapiens]	628	614/614 (100)	614/614 (100)	0.0
gi|13751645|emb|CAC37289.1| (AL031258)	C335H7.1 (mesothelin) [Homo sapiens]	630	590/605 (97)	594/605 (97)	0.0
gi|5031917|ref|NP_005814.1| (NM_005823)	megakaryocyte potentiating factor precursor; mesothelin isoform 1 precursor; megakaryocyte potentiating factor [Homo sapiens]	622	583/605 (96)	585/605 (96)	0.0
gi|14336721|gb|AAK61253.1| AE006464_21 (AE006464)	pre-pro-megakarycyte potentiating factor precursor [Homo sapiens]	622	582/605 (96)	586/605 (96)	0.0
gi|14424505|gb|AAH09272.1| AAH09272 (BC009272)	Unknown (protein for MGC:10273) [Homo sapiens]	622	581/605 (96)	585/605 (96)	0.0

This BLASTP data is displayed graphically in the ClustalW in Table 2E. A multiple
sequence alignment is given in Table 2E, with the SEC2 protein being shown on line 1, in a
ClustalW analysis comparing the protein of the invention with the related protein sequences
shown in Table 2D.

<table>
<tr><td colspan="2">Table 2E. ClustalW Alignment of SEC2</td></tr>
</table>

```
1)  SEC2 (SEQ ID NO:4)
2)  gi|7108356|    (SEQ ID NO:46)
3)  gi|13751645|   (SEQ ID NO:47)
4)  gi|5031917|    (SEQ ID NO:48)
5)  gi|14336721|   (SEQ ID NO:49)
6)  gi|14424505|   (SEQ ID NO:50)

                        10        20        30        40        50
               ....|....|....|....|....|....|....|....|....|....|
SEC2           MALQRLDPCW-SCGD-RPGSLLFLLFSLGWVHPARTLAGETGTESAPLGG
gi|7108356|    MALQRLDPCW-SCGD-RPGSLLFLLFSLGWVHPARTLAGETGTESAPLGG
gi|13751645|   MALPTARPLLGSCGTPALGSLLFLLFSLGWVQPSRTLAGETGQEAAPLDG
gi|5031917|    MALPTARPLLGSCGTPALGSLLFLLFSLGWVQPSRTLAGETGQEAAPLDG
gi|14336721|   MALPTARPLLGSCGTPALGSLLFLLFSLGWVQPSRTLAGETGQEAAPLDG
gi|14424505|   MALPTARPLLGSCGTPALGSLLFLLFSLGWVQPSRTLAGETGQEAAPLDG

                        60        70        80        90       100
               ....|....|....|....|....|....|....|....|....|....|
SEC2           VLTTPHNISSLSPRQLLGFPCAEVSGLSTERVRELAVALAQKNVKLSTEQ
gi|7108356|    VLTTPHNISSLSPRQLLGFPCAEVSGLSTERVRELAVALAQKNVKLSTEQ
gi|13751645|   VLANPPNISSLSPRQLLGFPCAEVSGLSTERVRELAVALAQKNVKLSTEQ
gi|5031917|    VLANPPNISSLSPRQLLGFPCAEVSGLSTERVRELAVALAQKNVKLSTEQ
gi|14336721|   VLANPPNISSLSPRQLLGFPCAEVSGLSTERVRELAVALAQKNVKLSTEQ
gi|14424505|   VLANPPNISSLSPRQLLGFPCAEVSGLSTERVRELAVALAQKNVKLSTEQ

                       110       120       130       140       150
               ....|....|....|....|....|....|....|....|....|....|
SEC2           LRCLAHRLSEPPEDLDALPLDLLLFLNPDAFSGPQACTRFFSRITKANVD
gi|7108356|    LRCLAHRLSEPPEDLDALPLDLLLFLNPDAFSGPQACTRFFSRITKANVD
gi|13751645|   LRCLAHRLSEPPEDLDALPLDLLLFLNPDAFSGPQACTRFFSRITKANVD
gi|5031917|    LRCLAHRLSEPPEDLDALPLDLLLFLNPDAFSGPQACTRFFSRITKANVD
gi|14336721|   LRCLAHRLSEPPEDLDALPLDLLLFLNPDAFSGPQACTRFFSRITKANVD
gi|14424505|   LRCLAHRLSEPPEDLDALPLDLLLFLNPDAFSGPQACTRFFSRITKANVD

                       160       170       180       190       200
               ....|....|....|....|....|....|....|....|....|....|
SEC2           LLPRGAPERQRLLPAALACWGVRGSLLSEADVRALGGLACDLPGRFVAES
gi|7108356|    LLPRGAPERQRLLPAALACWGVRGSLLSEADVRALGGLACDLPGRFVAES
gi|13751645|   LLPRGAPERQRLLPAALACWGVRGSLLSEADVRALGGLACDLPGRFVAES
gi|5031917|    LLPRGAPERQRLLPAALACWGVRGSLLSEADVRALGGLACDLPGRFVAES
gi|14336721|   LLPRGAPERQRLLPAALACWGVRGSLLSEADVRALGGLACDLPGRFVAES
gi|14424505|   LLPRGAPERQRLLPAALACWGVRGSLLSEADVRALGGLACDLPGRFVAES

                       210       220       230       240       250
               ....|....|....|....|....|....|....|....|....|....|
SEC2           AEVLLPRLVSCPGPLDQDQQEAARAALQGGGPPYGPPSTWSVSTMDALRG
gi|7108356|    AEVLLPRLVSCPGPLDQDQQEAARAALQGGGPPYGPPSTWSVSTMDALRG
gi|13751645|   AEVLLPRLVSCPGPLDQDQQEAARAALQGGGPPYGPPSTWSVSTMDALRG
gi|5031917|    AEVLLPRLVSCPGPLDQDQQEAARAALQGGGPPYGPPSTWSVSTMDALRG
gi|14336721|   AEVLLPRLVSCPGPLDQDQQEAARAALQGGGPPYGPPSTWSVSTMDALRG
gi|14424505|   AEVLLPRLVSCPGPLDQDQQEAARAALQGGGPPYGPPSTWSVSTMDALRG

                       260       270       280       290       300
               ....|....|....|....|....|....|....|....|....|....|
```

```
SEC2            LLPVLGQPIIRSIPQGIVAAWRQRSSRDPSWRQPERTILRPRFRREVEKT
gi|7108356|     LLPVLGQPIIRSIPQGIVAAWRQRSSRDPSWRQPERTILRPRFRREVEKT
gi|13751645|    LLPVLGQPIIRSIPQGIVAAWRQRSSRDPSWRQPERTILRPRFRREVEKT
gi|5031917|     LLPVLGQPIIRSIPQGIVAAWRQRSSRDPSWRQPERTILRPRFRREVEKT
gi|14336721|    LLPVLGQPIIRSIPQGIVAAWRQRSSRDPSWRQPERTILRPRFRREVEKT
gi|14424505|    LLPVLGQPIIRSIPQGIVAAWRQRSSRDPSWRQPERTILRPRFRREVEKT

                        310       320       330       340       350
                ....|....|....|....|....|....|....|....|....|....|
SEC2            ACPSGKKAREIDESLIFYKKWELEACVDAALLATQMDRVNAIPFTYEQLD
gi|7108356|     ACPSGKKAREIDESLIFYKKWELEACVDAALLATQMDRVNAIPFTYEQLD
gi|13751645|    ACPSGKKAREIDESLIFYKKWELEACVDAALLATQMDRVNAIPFTYEQLD
gi|5031917|     ACPSGKKAREIDESLIFYKKWELEACVDAALLATQMDRVNAIPFTYEQLD
gi|14336721|    ACPSGKKAREIDESLIFYKKWELEACVDAALLATQMDRVNAIPFTYEQLD
gi|14424505|    ACPSGKKAPEIDESLIFYKKWELEACVDAALLATQMDRVNAIPFTYEQLD

                        360       370       380       390       400
                ....|....|....|....|....|....|....|....|....|....|
SEC2            VLKHKLDELYPQGYPESVIQHLGYLFLKMSPEDIRKWNVTSLETLKALLE
gi|7108356|     VLKHKLDELYPQGYPESVIQHLGYLFLKMSPEDIRKWNVTSLETLKALLE
gi|13751645|    VLKHKLDELYPQGYPESVIQHLGYLFLKMSPEDIRKWNVTSLETLKALLE
gi|5031917|     VLKHKLDELYPQGYPESVIQHLGYLFLKMSPEDIRKWNVTSLETLKALLE
gi|14336721|    VLKHKLDELYPQGYPESVIQHLGYLFLKMSPEDIRKWNVTSLETLKALLE
gi|14424505|    VLKHKLDELYPQGYPESVIQHLGYLFLKMSPEDIRKWNVTSLETLKALLE

                        410       420       430       440       450
                ....|....|....|....|....|....|....|....|....|....|
SEC2            VDKGHEMSPQAPRRPLPQVATLIDRFVKGRGQLDKDTLDTLTAFYPGYLC
gi|7108356|     VDKGHEMSPQAPRRPLPQVATLIDRFVKGRGQLDKDTLDTLTAFYPGYLC
gi|13751645|    VNKGHEMSPQAPRRPLPQVATLIDRFVKGRGQLDKDTLDTLTAFYPGYLC
gi|5031917|     VNKGHEMS--------PQVATLIDRFVKGRGQLDKDTLDTLTAFYPGYLC
gi|14336721|    VNKGHEMS--------PQVATLIDRFVKGRGQLDKDTLDTLTAFYPGYLC
gi|14424505|    VNKGHEMS--------PQVATLIDRFVKGRGQLDKDTLDTLTAFYPGYLC

                        460       470       480       490       500
                ....|....|....|....|....|....|....|....|....|....|
SEC2            SLSPEELSSVPPSSIWAVRPQDLDTCDPRQLDVLYPKARLAFQNMNGSEY
gi|7108356|     SLSPEELSSVPPSSIWAVRPQDLDTCDPRQLDVLYPKARLAFQNMNGSEY
gi|13751645|    SLSPEELSSVPPSSIWAVRPQDLDTCDPRQLDVLYPKARLAFQNMNGSEY
gi|5031917|     SLSPEELSSVPPSSIWAVRPQDLDTCDPRQLDVLYPKARLAFQNMNGSEY
gi|14336721|    SLSPEELSSVPPSSIWAVRPQDLDTCDPRQLDVLYPKARLAFQNMNGSEY
gi|14424505|    SLSPEELSSVPPSSIWAVRPQDLDTCDPRQLDVLYPKARLAFQNMNGSEY

                        510       520       530       540       550
                ....|....|....|....|....|....|....|....|....|....|
SEC2            FVKIQSFLGGAPTEDLKALSQQNVSMDLATFMKLRTDAVLPLTVAEVQKL
gi|7108356|     FVKIQSFLGGAPTEDLKALSQQNVSMDLATFMKLRTDAVLPLTVAEVQKL
gi|13751645|    FVKIQSFLGGAPTEDLKALSQQNVSMDLATFMKLRTDAVLPLTVAEVQKL
gi|5031917|     FVKIQSFLGGAPTEDLKALSQQNVSMDLATFMKLRTDAVLPLTVAEVQKL
gi|14336721|    FVKIQSFLGGAPTEDLKALSQQNVSMDLATFMKLRTDAVLPLTVAEVQKL
gi|14424505|    FVKIQSFLGGAPTEDLKALSQQNVSMDLATFMKLRTDAVLPLTVAEVQKL

                        560       570       580       590       600
                ....|....|....|....|....|....|....|....|....|....|
SEC2            LGPHVEGLKAEERHRPVRDWILRQRQDDLDTLGLGLQGGIPNGYLVLDLS
gi|7108356|     LGPHVEGLKAEERHRPVRDWILRQRQDDLDTLGLGLQGGIPNGYLVLDLS
gi|13751645|    LGPHVEGLKAEERHRPVRDWILRQRQDDLDTLGLGLQGGIPNGYLVLDLS
gi|5031917|     LGPHVEGLKAEERHRPVRDWILRQRQDDLDTLGLGLQGGIPNGYLVLDLS
gi|14336721|    LGPHVEGLKAEERHRPVRDWILRQRQDDLDTLGLGLQGGIPNGYLVLDLS
gi|14424505|    LGPHVEGLKAEERHRPVRDWILRQRQDDLDTLGLGLQGGIPNGYLVLDLS

                        610       620       630
                ....|....|....|....|....|....|.
SEC2            VQETLSGTPCLLGPGPVLTVLALLLASTLA
gi|7108356|     VQETLSGTPCLLGPGPVLTVLALLLASTLA
gi|13751645|    MQEALSGTPCLLGPGPVLTVLALLLASTLA
gi|5031917|     VQEALSGTPCLLGPGPVLTVLALLLASTLA
gi|14336721|    MQEALSGTPCLLGPGPVLTVLALLLASTLA
gi|14424505|    MQEALSGTPCLLGPGPVLTVLALLLASTLA
```

21

Mesothelin, a cell-surface differentiation antigen, is a 40-kD GPI-linked (glycosylphosphatidylinositol) cell-surface glycoprotein, that is present on the surface of normal mesothelium and is overexpressed in many patients with cancer and malignant

5   mesotheliomas. For example, mesothelin is a marker for pancreatic adenocarcinoma, ovarian cancer, pancreatic cancer, lung cancer, squamous cell carcinoma, and numerous other neoplastic cellular transformations as identified by gene expression analysis. Mesothelin overexpression in cancers has potential diagnostic, imaging, and therapeutic implications. Mesothelin is a antigen that is expressed in a highly tissue-specific manner. High level

10   expression of the protein is seen in the mesothelium, and the tissue forming the pleural, pericardial, and peritoneal membranes. The gene contains an 1884-bp open reading frame encoded by 15 exons occupying 8 kb of human chromosome 16. An 1850-bp region of genomic DNA at the 5' end of the gene encompassing the proposed transcriptional start site was also cloned. This region lacks a TATA box and other regulatory elements such as SP1

15   sites, which are commonly found in promoters. Transient transfection analyses demonstrated that mesothelium-specific control elements are present within the 1.85-kb region. Minimal constitutive promoter elements were localized to a 317-bp region. Tissue-specific enhancer elements upstream of the minimal promoter were found to activate transcription from the homologous and a heterologous promoter in a position- and orientation-independent manner.

20   The SEC2 disclosed in this invention is expressed in at least the following tissues: apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye,

25   frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, those that express MHC I, II, and III, nervous tissues, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortia) spinal cord, spleen, stomach, taste receptor cells of

30   the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, public EST sources, literature sources, and/or RACE sources. Further expression data for SEC2 is provided in Example 2.

The nucleic acids and proteins of SEC2 are useful in potential therapeutic applications implicated in various SEC-related pathological disorders described further herein. The SEC2 nucleic acid encoding the mesothelin-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid

5   or the protein are to be assessed.

These materials are further useful in the generation of antibodies that bind immunospecifically to the substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-SECX Antibodies" section

10   below. The disclosed SEC2 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, for example, a contemplated SEC2 epitope comprises from about amino acids 5 to about 105. In another embodiment, for example, a SEC2 epitope comprises from about amino acids 120 to about 180. In further embodiments, for example, a SEC2 epitope comprises from about 210 to about 230, from about 260 to about

15   310, from about 320 to about 420, from about 450 to about 520, and about 550 to about 600.

SEC3

The disclosed SEC3 (alternatively referred to herein as CG56015-01) includes the 852 nucleotide sequence (SEQ ID NO:5) shown in Table 3A. A SEC3 ORF begins with a Kozak

20   consensus ATG initiation codon at nucleotides 131-133 and ends with a TGT codon at nucleotides 471-473. A putative untranslated region upstream from the initiation codon is underlined in Table 3A, and the start and stop codons are in bold letters.

Table 3A. SEC3 Nucleotide Sequence (SEQ ID NO:5)
CTTCCTAGCTCCTCTCCTCCAGGGCCAGACTGAGCCCAGGTTGATTTCAGGCGGACACCA ATAGACTCCACAGCAGCTCCAGGAGCCCAGACACCGGCGGCCAGAAGCAAGGCTAGGAGC TGCTGCAGCC**ATG**TCGGCCCTCAGCCTCCTCATTCTGGGCCTGCTCACGGCAGTGCCACC TGCCAGCTGTCAGCAAGGCCTGGGGAACCTTCAGCCCTGGATGCAGGGCCTTATCGCGGT GGCCGTGTTCCTGGTCCTCGTTGCAATCGCCTTTGCAGTCAACCACTTCTGGTGCCAGGA GGAGCCGGAGCCTGCACACATGATCCTGACCGTCGGAAACAAGGCAGATGGAGTCCTGGT GGGAACAGATGGAAGGTACTCTTCGATGGCGGCCAGTTTCAGGTCCAGTGAGCATGAGAA TGCCTATGAGAATGTGCCCGAGGAGGAAGGCAAGGTCCGCAGCACCCC**GATGTAA**CCTTC TCTGTGGCTCCAACCCCAAGACTCCCAGGCACATGGGATGGATGTCCAGTGCTACCACCC AAGCCCCCTCCTTCTTTGTGTGGAATCTGCAATAGTGGGCTGACTCCCTCCAGCCCCATG CCGGCCCTACCCGCCCTTGAAGTATAGCAGCCAAGGTTGGAGCTCAGACCGTGTCTAGG TTGGGGCTCGGCTGTGGCCCTGGGGTCTCCTGCTCAGCTCAGAAGAGCCTTCTGGAGAGG ACAGTCAGCTGAGCACCTCCCATCCTGCTCACACGTCCTTCCCCATAACTATGGAAATGG CCCTAATTTCTGTGAAATAAAGACTTTTTGTATTTCTGGGGCTGAGGCTCAGCAACAGCC CCTCAGGCTTCC

The SEC3 polypeptide (SEQ ID NO:6) encoded by SEQ ID NO:5 is 114 amino acids in length and is presented using the one-letter amino acid code in Table 3B. The Psort profile for SEC3 predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.4600. In alternative embodiments, a SEC3 polypeptide is located to endoplasmic reticulum (membrane) with a certainty of 0.1000, or to the endoplasmic reticulum (lumen) with a certainty of 0.1000. The Signal P predicts a likely cleavage site for a SEC3 peptide is between positions 49 and 50, *i.e.*, at the dash in the sequence AFA-VN.

Table 3B. SEC3 protein sequence (SEQ ID NO:6)
MSALSLLILGLLTAVPPASCQQGLGNLQPWMQGLIAVAVFLVLVAIAFAVNHFWCQEEPE PAHMILTVGNKADGVLVGTDGRYSSMAASFRSSEHENAYENVPEEEGKVRSTPM

A BLAST analysis of SEC3 was run against the proprietary PatP GENESEQ Protein Patent database. It was found, for example, that the amino acid sequence of SEC3 had high homology to other proteins as shown in Table 3C.

Table 3C. BLASTX results from PatP database for SEC3		
Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum Probability P(N)
patp:AAG00220 Human secreted protein	394	2.2e-36
patp:AAY13947 Human transmembrane protein, HP10495	92	0.00022
patp:AAY07878 Human secreted protein fragment	92	0.00022
patp:AAY11997 Human 5' EST secreted protein	92	0.00022
patp:AAG73549 Human colon cancer antigen protein	72	0.067

In a search of public sequence databases, it was found, for example, that the amino acid sequence of the SEC3 protein of the present invention was found to have 114 of 114 amino acid residues (100%) identical to the 104 amino acid NM_005764. SEC3 also has homology to the other proteins shown in the BLASTP data in Table 3D.

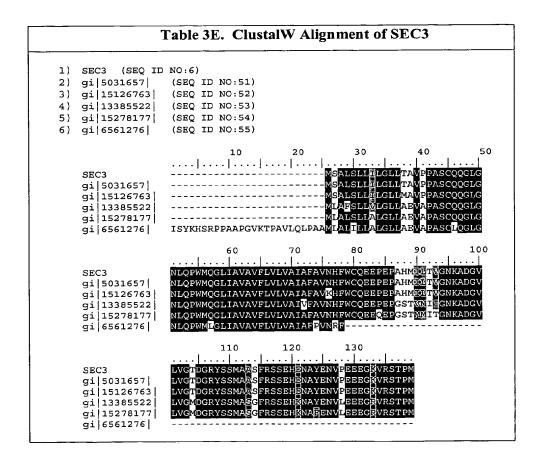| Table 3D. SEC3 BLASTP results					
Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positive (%)	Expect
gi|5031657|ref|NP_005755.1| (NM_005764)	epithelial protein up-regulated in carcinoma, membrane associate [Homo sapiens]	114	114/114 (100)	114/114 (100)	6e-53

gi|15126763|g b|AAH12303.1| AAH12303 (BC012303)	Similar to epithelial protein up-regulated in carcinoma, membrane associated protein 17 [Homo sapiens]	114	112/114 (98)	112/114 (98)	1e-51
gi|13385522|r ef|NP_080294. 1| (NM_026018)	RIKEN cDNA 2700030M23 gene [Mus musculus]	114	94/114 (82)	101/114 (88)	2e-48
gi|15278177|g b|AAK94063.1| AF402772_1 (AF402772)	membrane-associated protein MAP17 [Rattus norvegicus]	114	94/114 (82)	100/114 (87)	2e-42
gi|6561276|gb |AAF16875.1| (AF110026)	DD96 homolog [Rattus norvegicus]	78	43/53 (81)	43/53 (81)	2e-09

This BLASTP data is displayed graphically in the ClustalW in Table 3E. A multiple sequence alignment is given in Table 3E, with the SEC3 protein being shown on line 1, in a ClustalW analysis comparing the protein of the invention with the related protein sequences shown in Table 3D.

```
              Table 3E.  ClustalW Alignment of SEC3

     1)  SEC3   (SEQ ID NO:6)
     2)  gi|5031657|    (SEQ ID NO:51)
     3)  gi|15126763|   (SEQ ID NO:52)
     4)  gi|13385522|   (SEQ ID NO:53)
     5)  gi|15278177|   (SEQ ID NO:54)
     6)  gi|6561276|    (SEQ ID NO:55)

                       10        20        30        40        50
               ....|....|....|....|....|....|....|....|....|....|
     SEC3          ----------------------------MSALSLLILGLLTAVPPASCQQGLG
     gi|5031657|   ----------------------------MSALSLLILGLLTAVPPASCQQGLG
     gi|15126763|  ----------------------------MSALSLLILGLLMAVPPASCQQGLG
     gi|13385522|  ----------------------------MLAFSLLVLGLLAEVAPASCQQGLG
     gi|15278177|  ----------------------------MLALSLIALGLLAEVAPASCQQGLG
     gi|6561276|   ISYKHSRPPAAPGVKTPAVLQLPAAMLAIILLALGLLAEVAPASCLQGLG

                       60        70        80        90       100
               ....|....|....|....|....|....|....|....|....|....|
     SEC3          NLQPWMQGLIAVAVFLVLVAIAFAVNHFWCQEEPEPAHMILTVGNKADGV
     gi|5031657|   NLQPWMQGLIAVAVFLVLVAIAFAVNHFWCQEEPEPAHMILTVGNKADGV
     gi|15126763|  NLQPWMQGLIAVAVFLVLVAIAFAVKHFWCQEEPEPAHMILTVGNKADGV
     gi|13385522|  NLQPWMQGLIAVAVFLVLVAIVFAVNHFWCQEEPEPGSTVMIIGNKADGV
     gi|15278177|  NLQPWMQGLIAVAVFLVLVAIAFAVNHFWCQEEQEPGSTMMITGNKADGV
     gi|6561276|   NLQPWMLGLIAVAVFLVLVAIAFPVNRF---------------------

                      110       120       130
               ....|....|....|....|....|....|....|....
     SEC3          LVGTDGRYSSMAASFRSSEHENAYENVPEEEGKVRSTPM
     gi|5031657|   LVGTDGRYSSMAASFRSSEHENAYENVPEEEGKVRSTPM
     gi|15126763|  LVGTDGRYSSMAASFRSSEHENAYENVPEEEGKVRSTPM
     gi|13385522|  LVGMDGRYSSMASGFRSSEHKNAYENVLEEEGRVRSTPM
     gi|15278177|  LVGMDGRYSSMASGFRSSEHKNAFENVLEEEGRVRSTPM
     gi|6561276|   ---------------------------------------
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MAP17 and PDZK1 mRNAs are markedly up-regulated in human carcinomas. The MAP17 protein product is a 17-kd membrane-associated protein as determined by

immunoprecipitation. MAP17 is expressed at significant levels in a the proximal tubular epithelial cells of the kidney, and is induced in immortalized breast ductal epithelial cell lines compared with normal breast ductal epithelial cells, and, *in vivo*, in premalignant conditions, such as adenoma of the colon and ductal carcinoma *in situ* of the breast. MAP17 is expressed

5   abundantly in carcinomas arising from kidney, colon, lung, and breast, in some cases with a membrane-associated apical glandular distribution. In tissue culture, MAP17 was localized to the cell membrane in areas of cell-cell contact, i.e., the distribution of cell-function-associated proteins. Transfection of a full-length wild-type MAP17 cDNA clone into a colon carcinoma cell line, HT-29, markedly decreased cell proliferation *in vitro* and tumor growth *in vivo*.

10   MAP17 plays a role in the early events associated with malignant transformation, and modulation of MAP17 expression and activation thus provides for a method of treating the above mentioned diseases.

The MAP17 disclosed herein as SEC3 in this invention is expressed in at least the following tissues: apical microvilli of the retinal pigment epithelium, arterial (aortic), basal

15   forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult

20   lymphoid tissue, those that express MHC I, II, and III, nervous tissues, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortia) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention

25   including but not limited to SeqCalling sources, public EST sources, literature sources, and/or RACE sources. Further expression data for SEC3 is provided in Example 2.

The nucleic acids and proteins of SEC3 are useful in potential therapeutic applications implicated in various SEC-related pathological disorders described further herein. The SEC3 nucleic acid encoding the MAP17-like protein of the invention, or fragments thereof, may

30   further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

These materials are further useful in the generation of antibodies that bind immunospecifically to the substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using

26

prediction from hydrophobicity charts, as described in the "Anti-SECX Antibodies" section below. The disclosed SEC3 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, for example, a contemplated SEC3 epitope comprises from about amino acids 20 to about 28. In another embodiment, for example, a

5   SEC3 epitope comprises from about amino acids 55 to about 65. In further embodiments, for example, a SEC3 epitope comprises from about 70 to about 80, and from about 82 to about 114.

SEC4

10   The disclosed SEC4 (alternatively referred to herein as CG55023-01) includes the 527 nucleotide sequence (SEQ ID NO:7) shown in Table 4A. A SEC4 ORF begins with a Kozak consensus ATG initiation codon at nucleotides 59-61 and ends with a TGT codon at nucleotides 509-511. A putative untranslated region upstream from the initiation codon is underlined in Table 4A, and the start and stop codons are in bold letters. The disclosed SEC4

15   maps to chromosome 20.

Table 4A. SEC4 Nucleotide Sequence (SEQ ID NO:7)
CCGTCAGTCTAGAAGGATAAGAGAAAGAAAGTTAAGCAACTACAGGAAATGGCTTTGGGA GTTCCAATATCAGTCTATCTTTTATTCAACGCAATGACAGCACTGACCGAAGAGGCAGCC GTGACTGTAACACCTCCAATCACAGCCCAGCAAGGTAACTGGACAGTTAACAAAACAGAA GCTCACAACATAGAAGGACCCATAGCCTTGAAGTTCTCACACCTTTGCCTGGAAGATCAT AACAGTTACTGCATCAACGGTGCTTGTGCATTCCACCATGAGCTAGAGAAAGCCATCTGC AGGTGTTTTACTGGTTATACTGGAGAAAGGTGTGAGCACTTGACTTTAACTTCATATGCT GTGGATTCTTATGAAAAATACATTGCAATTGGGATTGGTGTTGGATTACTATTAAGTGGT TTTCTTGTTATTTTTTACTGCTATATAAGAAAGAGGTGTCTAAAATTGAAATCGCCTTAC AATGTCTGTTCTGGAGAAAGACGACCACTGTGAGGCCTTTGTGAAGA

The SEC4 polypeptide (SEQ ID NO:8) encoded by SEQ ID NO:7 is 154 amino acids in length and is presented using the one-letter amino acid code in Table 4B. The Psort profile

20   for SEC4 predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.4960. In alternative embodiments, a SEC4 polypeptide is located to the Golgi body with a certainty of 0.1900, to the endoplasmic reticulum (membrane) with a certainty of 0.6400, or to the endoplasmic reticulum (lumen) with a certainty of 0.1000. The Signal P predicts a likely cleavage site for a SEC4 peptide is

25   between positions 20 and 21, i.e., at the dash in the sequence ALT-EE.

Table 4B. SEC4 protein sequence (SEQ ID NO:8)
MALGVPISVYLLFNAMTALTEEAAVTVTPPITAQQGNWTVNKTEAHNIEGPIALKFSHLC LEDHNSYCINGACAFHHELEKAICRCFTGYTGERCEHLTLTSYAVDSYEKYIAIGIGVGL LLSGFLVIFYCYIRKRCLKLKSPYNVCSGERRPL

A BLAST analysis of SEC4 was run against the proprietary PatP GENESEQ Protein Patent database. It was found, for example, that the amino acid sequence of SEC4 had high homology to other proteins as shown in Table 4C.

5

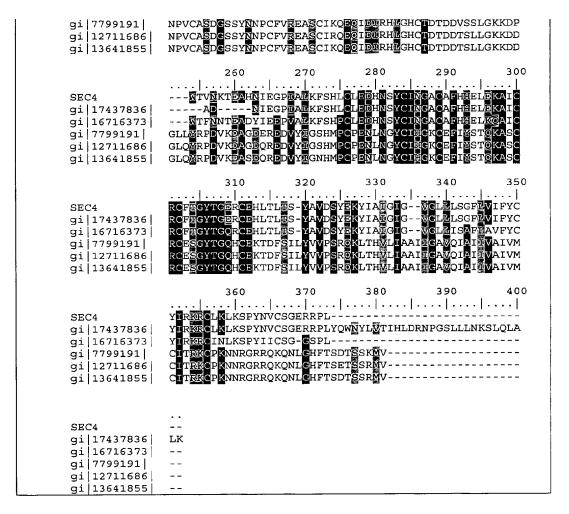| Table 4C. BLASTX results from PatP database for SEC4		
Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum Probability P(N)
patp:AAY76018 Human TGF-alpha homologue huTR1	828	2.2e-82
patp:AAB55957 Skin cell protein	828	2.2e-82
patp:AAE06704 Human transforming growth factor (TGF)alpha	828	2.2e-82
patp:AAY94620 Epidermal growth factor-like variant	819	2.0e-81
patp:AAY76009 Murine TGF-alpha homologue muTR1	629	2.8e-61

In a search of public sequence databases, it was found, for example, that the amino acid sequence of the SEC4 protein of the present invention was found to have 129 of 139 amino acid residues (92%) identical to the 159 amino acid XM_068181. SEC4 also has

10   homology to the other proteins shown in the BLASTP data in Table 4D.

Table 4D. SEC4 BLASTP results					
Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positive (%)	Expect
gi|17437836|r ef|XP_068181. 1| (XM_068181)	similar to Epigen protein (H. sapiens) [Homo sapiens]	159	129/139 (92)	129/139 (92)	3e-66
gi|16716373|r ef|NP_444317. 1| (NM_053087)	epithelial mitogen; RIKEN cDNA 2310069M11 gene [Mus musculus]	152	119/150 (79)	129/150 (85)	1e-59
gi|7799191|em b|CAB90827.1| (AJ400622)	tomoregulin-1 [Mus musculus]	354	32/87 (36)	48/87 (54)	7e-07
gi|12711686|r ef|NP_075409. 1| (NM_023020)	transmembrane protein with EGF-like and two follistatin-like domains 1 [Rattus norvegicus]	373	32/87 (36)	48/87 (54)	8e-07

gi|13641855|ref|XP_005346.3| (XM_005346)	similar to transmembrane protein with EGF-like and two follistatin-like domains 1; chromosome 9 open reading frame 2 (H. sapiens) [Homo sapiens]	53	30/83 (36)	45/83 (54)	1e-06

This BLASTP data is displayed graphically in the ClustalW in Table 4E. A multiple sequence alignment is given in Table 4E, with the SEC4 protein being shown on line 1, in a ClustalW analysis comparing the protein of the invention with the related protein sequences shown in Table 4D.

```
                    Table 4E.  ClustalW Alignment of SEC4

  1)  SEC4   (SEQ ID NO:8)
  2)  gi|17437836|   (SEQ ID NO:56)
  3)  gi|16716373|   (SEQ ID NO:57)
  4)  gi|7799191|    (SEQ ID NO:58)
  5)  gi|12711686|   (SEQ ID NO:59)
  6)  gi|13641855|   (SEQ ID NO:60)

                         10        20        30        40        50
                ....|....|....|....|....|....|....|....|....|....|
  SEC4          --------------------------------------------------
  gi|17437836|  --------------------------------------------------
  gi|16716373|  --------------------------------------------------
  gi|7799191|   --------------------YPQDSARVAFCLPGSRASNQPAGGGG--
  gi|12711686|  MG---AQAPLRLPAAPPLAVCGYTSVLLLFAFCLPGSGASNQPAGGGG--
  gi|13641855|  MGAAAAEAPLRLPAAPPLAFCCYTSVLLLFAFSLPGSRASNQPPGGGGGS

                         60        70        80        90       100
                ....|....|....|....|....|....|....|....|....|....|
  SEC4          --------------------------------------------------
  gi|17437836|  --------------------------------------------------
  gi|16716373|  --------------------------------------------------
  gi|7799191|   --DCPGGRGKS-NCSELNLRESDIRVCDESSCKYGGVCKEDGDGLKCACQ
  gi|12711686|  --DCPGGRGKSINCSELNLRESDIRACDESSCKYGGVCKEDGDGLKCACQ
  gi|13641855|  GGDCPGGKGKSINCSELNVRESDVRVCDESSCKYGGVCKEDGDGLKCACQ

                        110       120       130       140       150
                ....|....|....|....|....|....|....|....|....|....|
  SEC4          --------------------------------------------------
  gi|17437836|  --------------------------------------------------
  gi|16716373|  --------------------------------------------------
  gi|7799191|   FQCHTNYIPVCGSNGDTYQNECFLRRAACKHQKDITVVARGPCYSDNGSG
  gi|12711686|  FQCHTNYIPVCGSNGDTYQNECFLRRAACKHQKDITVVARGPCYSDNGSG
  gi|13641855|  FQCHTNYIPVCGSNGDTYQNECFLRRAACKHQKEITVIARGPCYSDNGSG

                        160       170       180       190       200
                ....|....|....|....|....|....|....|....|....|....|
  SEC4          --------------------------------------------------
  gi|17437836|  --------------------------------------------------
  gi|16716373|  --------------------------------------------------
  gi|7799191|   SGEGAEEEGSGAGAHRKHSKCGPCKYKAECDEDAENVGCVCNIDCSGYSF
  gi|12711686|  SGEG-EEEGSGAGAHRKHSKCGPCKYKAECDEDAENVGCVCNIDCSGYSF
  gi|13641855|  SGEG-EEEGSGAEVHRKHSKCGPCKYKAECDEDAENVGCVCNIDCSGYSF

                        210       220       230       240       250
                ....|....|....|....|....|....|....|....|....|....|
  SEC4          ----MALGVPISVYLLFNAMT-ALTEEAAVTVTPPITAQQGN--------
  gi|17437836|  ------------------MT-ALTEEAAVTVTPPITAQQ----------
  gi|16716373|  ----MALGVLIAVCLLFKAMKAALSEEAEV--IPPSTAQQSN--------
```

```
gi|7799191|     NPVCASDGSSYNNPCFVREASCIKQEQIDIRHIGHCIDTDDVSSLGKKDP
gi|12711686|    NPVCASDGSSYNNPCFVREASCIRQEQIDIRHIGHCIDTDDTSLLGKKDD
gi|13641855|    NPVCASDGSSYNNPCFVREASCIKQEQIDIRHIGHCIDTDDTSLLGKKDD

                        260       270       280       290       300
                ....|....|....|....|....|....|....|....|....|....|
SEC4            ---WTVNKTEAHNIEGPIAIKFSHLCIEDHNSYCINGACAFHIELEKAIC
gi|17437836|    -----AD-----NIEGPIAIKFSHLCIEDHNSYCINGACAFHIELEKAIC
gi|16716373|    ---WTFNNTEADYIEBPVAIKFSHPCIEDHNSYCINGACAFHIELKQAIC
gi|7799191|     GLLWRPDVKDAGBEREDVYTGSHMPCPBNLNGYCIBGKCEFIYSTQKASC
gi|12711686|    GLQWRPDVKDAGDQREDVYTGSHMPCPBNLNGYCIBGKCEFIYSTQKASC
gi|13641855|    GLQWRPDVKDASEQREDVYTGNHMPCPBNLNGYCIBGKCEFIYSTQKASC

                        310       320       330       340       350
                ....|....|....|....|....|....|....|....|....|....|
SEC4            RCFIGYTGERCBHLTLTS-YAVDSYEKYIAIGIG--VGLILLSGFLVIFYC
gi|17437836|    RCFIGYTGERCBHLTLIS-YAVDSYEKYIAIGIG--VGLILLSGFLVIFYC
gi|16716373|    RCFIGYTGQRCBHLTLIS-YAVDSYEKYIAIGIG--VGLLISAFIAVFYC
gi|7799191|     RCESGYTGOHCBKTDFSILYVVPSRQKLTHVLIAAIIGAVQIAIIVAIVM
gi|12711686|    RCESGYTGOHCBKTDFSILYVVPSRQKLTHVLIAAIIGAVQIAIIVAIVM
gi|13641855|    RCESGYTGQHCBKTDFSILYVVPSRQKLTHVLIAAIIGAVQIAIIVAIVM

                        360       370       380       390       400
                ....|....|....|....|....|....|....|....|....|....|
SEC4            YIRKFCLKLKSPYNVCSGERRPL-------------------------
gi|17437836|    YIRKFCLKLKSPYNVCSGERRPLYQWNYLVTIHLDRNPGSLLLNKSLQLA
gi|16716373|    YIRKFCINLKSPYIICSG-GSPL-------------------------
gi|7799191|     CITRKFCPKNNRGRRQKQNLGHFTSDTSSKMV-------------------
gi|12711686|    CITRKFCPKNNRGRRQKQNLGHFTSETSSRMV-------------------
gi|13641855|    CITRKFCPKNNRGRRQKQNLGHFTSDTSSRMV-------------------


SEC4            --
gi|17437836|    LK
gi|16716373|    --
gi|7799191|     --
gi|12711686|    --
gi|13641855|    --
```

The biological effects of epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-alpha) are mediated by an interaction with a specific cell surface receptor having both intra- and extracellular domains. The structure of the intracellular domain can be closely aligned with retroviral protein tyrosine kinases. Upon ligand-binding there is a change in conformation of the extracellular domain, the receptor being converted to dimeric. Dimeric receptor has a higher rate of catalysis than monomeric and rapidly becomes phosphorylated. This form of the receptor now associates with and phosphorylates enzymes such as phospholipase-C, altering their catalytic activity and subcellular distribution. This system appears to stimulate the effects of epidermal growth factor receptor (EGFr) activation, notably proliferation, morphology, paracrine effects and differentation. Coexpression of transforming growth factor alpha (TGF-alpha) and its receptor epidermal growth factor receptor (EGFR) is known to be associated with aggressive biologic behavior and adverse clinical outcome in a variety of tumors, for example, pancreatic adenocarcinomas.

The TGF-alpha precursor disclosed herein as SEC4 is expressed in at least the following tissues: apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical

5   neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, those that express MHC I, II, and III, nervous tissues, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle,

10   small intestine, smooth muscle (coronary artery in aortia) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, public EST sources, literature sources, and/or RACE sources. Further expression data for SEC4 is provided in Example 2.

15   The nucleic acids and proteins of SEC4 are useful in potential therapeutic applications implicated in various SEC-related pathological disorders described further herein. The SEC4 nucleic acid encoding the TGF alpha-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

20   These materials are further useful in the generation of antibodies that bind immunospecifically to the substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-SECX Antibodies" section below. The disclosed SEC4 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, for example, a

25   contemplated SEC4 epitope comprises from about amino acids 25 to about 50. In another embodiment, for example, a SEC4 epitope comprises from about amino acids 55 to about 75. In further embodiments, for example, a SEC4 epitope comprises from about 82 to about 105, and from about 135 to about 154.

30   **SEC5**

The disclosed SEC5 (alternatively referred to herein as CG56153-01) includes the 1293 nucleotide sequence (SEQ ID NO:9) shown in Table 5A. A SEC5 ORF begins with a Kozak consensus ATG initiation codon at nucleotides 144-146 and ends with a TAG codon at

nucleotides 1086-1088. A putative untranslated region upstream from the initiation codon is underlined in Table 5A, and the start and stop codons are in bold letters.

Table 5A. SEC5 Nucleotide Sequence (SEQ ID NO:9)
AGGTGGCGGGCGGGTACTTAAGGCGCGGCCACCGGGCTGGCAGTGCGCCCAACAGCGGAC
TCCGAGACCAGCGGATCTCGGCAAACCCTCTTTCTCGACCACCCACCTACCATTCTTGGA
ACCATGGCGGCAGTGGCGGCGGCCTCGGCTGAACTGCTCATCATCGGCTGGTACATCTTC
CGCGTGCTGCTGCAGGTGTTCCTGGAATGCTGCATTTACTGGGTAGGATTCGCTTTTCGA
AATCCTCCAGGGACACAGCCCATTGCGAGAAGTGAGGTGTTCAGGTACTCCCTGCAGAAG
CTGGCATACACGGTGTCGCGGACCGGGCGGCAGGTGTTGGGGGAGCGCAGGCAGCGAGCC
CCCAACTGAGGCCCCAGCTCCCAGCCCTGGGCGGCCGTATCATCAGGTGCTCCTGTGCAT
CTCGGCCAGCACGGGAGCCAGTGCCCGCGCAGGAATGTGGGGTCCCCTGTGTTCCCTCGCC
AGAGCACTTGGCAAGGTCAGTGAGGGGCCAGTAGACCCCCGGAGAAGCAGTACCGACAAT
GACGAAGATACCAGATCCCTTCCCAACCCCTTTGCACCGGTCCCACTAAGGGGCAGGGTC
GAGAGAGGAGGGGGGATAGGGGGAGCAGACCCTGAGATCTGGGCATAGGCACCGCATTCT
GATCTGGACAAAGTCGGGACAGCACCATCCCAGCCCCGAAGCCCGGGCCATGCCAGCAGG
CCCCACCATGGAAATCAAAACACCGCACCAGCCAGCAGAATGGACATTCTGACATCGCCA
GCCGACGCCCTGAATCTTGGTGCAGCACCCACCGCGTGCCTGTGTGGCGGGACTGGAGGG
CACAGTTGAGGAAGGAGGGTGGTTAAGAAATACAGTGGGGCCCTCTCGCTGTCCCTTGCC
CAGGGCACTTGTATTCCAGCCTCGCTGCATTTGCTCTCTCGATTGCCCCTTTCCTCCTAC
ATGCCTCCCAAGCCCACCCTACTCCAAAAGTAATGTGTCACTTGATTTGGAACTATTCAA
GCAGTAAAAGTAAATGAATCCCACCTTTACTAAAACACTTTCTCTGAACCCCCCTTGCCC
CTCACTGATCTTGCTTTTCCCTGGTCTCAGCAGTTGTGGTCAATATTGTGGTAATCGCTA
ATTGTACTGATTGTTTAAGTGTGCATTAGTTGTCTCTCCCCAGCTAGATTGTAAGCTCCT
GGAGGACAGGGACCACCTCTACAAAAAATAAAAAAAGTACCTCCCCTGTCTCGCACAGTG
TCCCAGGACCCTGCGGTGCAGTAGAGGCGCACC

The SEC5 polypeptide (SEQ ID NO:10) encoded by SEQ ID NO:9 is 81 amino acids in length and is presented using the one-letter amino acid code in Table 5B. The Psort profile for SEC5 predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6850. In alternative embodiments, a SEC5 polypeptide is located to the Golgi body with a certainty of 0.3700, to the endoplasmic reticulum (membrane) with a certainty of 0.6400, or to peroxisomal microbodies with a certainty of 0.3200. The Signal P predicts a likely cleavage site for a SEC5 peptide is between positions 37 and 38, i.e., at the dash in the sequence GFA-FR.

Table 5B. SEC5 protein sequence (SEQ ID NO:10)
MAAVAAASAELLIIGWYIFRVLLQVFLECCIYWVGFAFRNPPGTQPIARSEVFRYSLQKL AYTVSRTGRQVLGERRQRAPN

A BLAST analysis of SEC5 was run against the proprietary PatP GENESEQ Protein Patent database. It was found, for example, that the amino acid sequence of SEC5 had high homology to other proteins as shown in Table 5C.

Table 5C. BLASTX results from PatP database for SEC5

Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum Probability P(N)
patp:AAW79279 Human neuronatin amino acid sequence	419	4.9e-39
patp:AAR96044 Neuronatin-alpha - Rattus rattus	409	5.7e-38
patp:AAW79277 Rat neuronatin-alpha amino acid sequence	409	5.7e-38
patp:AAW37777 Human neuronatin amino acid sequence	152	2.4e-22
patp:AAR96045 Neuronatin-beta - Rattus rattus	142	2.6e-21

In a search of public sequence databases, it was found, for example, that the amino acid sequence of the SEC5 protein of the present invention was found to have 81 of 81 amino acid residues (100%) identical to the 81 amino acid NM_005386. SEC5 also has homology to the proteins shown in the BLASTP data in Table 5D.

Table 5D. SEC5 BLASTP results

Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positive (%)	Expect
gi|4885521|ref|NP_005377.1| (NM_005386)	neuronatin [Homo sapiens]	81	81/81 (100)	81/81 (100)	6e-32
gi|6754864|ref|NP_035053.1| (NM_010923)	neuronatin [Mus musculus]	81	80/81 (98)	81/81 (99)	6e-32
gi|16758386|ref|NP_446053.1| (NM_053601)	neuronatin; neuronatin alpha [Rattus norvegicus]	81	79/81 (97)	80/81 (98)	7e-32
gi|12833393|dbj|BAB22507.1| (AK003004)	putative [Mus musculus]	208	75/80 (93)	76/80 (94)	2e-30
gi|1083432|pir||S51082	neuronatin-1 - mouse	55	75/80 (93)	76/80 (94)	2e-13

This BLASTP data is displayed graphically in the ClustalW in Table 5E. A multiple sequence alignment is given in Table 5E, with the SEC5 protein being shown on line 1, in a ClustalW analysis comparing the protein of the invention with the related protein sequences shown in Table 5D.

Table 5E. ClustalW Alignment of SEC5

```
1)SEC5 (SEQ ID NO:10)
2)gi|4885521|   (SEQ ID NO:61)
3)gi|6754864|   (SEQ ID NO:62)
```

```
                4) gi|16758386|   (SEQ ID NO:63)
                5) gi|12833393|   (SEQ ID NO:64)
                6) gi|1083432|    (SEQ ID NO:65)

                                  10        20        30        40        50
                            ....|....|....|....|....|....|....|....|....|....|
                SEC5        -MAAVAAASAELLIIGWYIFRVLLQVFLECCIYWVGFAFRNPPGTQPIAR
                gi|4885521| -MAAVAAASAELLIIGWYIFRVLLQVFLECCIYWVGFAFRNPPGTQPIAR
                gi|6754864| -MAAVAAASAELLIIGWYIFRVLLQVFLECCIYWVGFAFRNPPGTQPIAR
                gi|16758386|-MAAVAAASAELLIIGWYIFRVLLQVFLECCIYWVGFAFRNPPGTQPIAR
                gi|12833393|-MAAVAAASAELLIIGWYIFRVLLQVFLECCIYWVGFAFRNPPGTQPIAR
                gi|1083432| MVPKVAAASAELLIIGWYIFRVLLQ------------------------

                                  60        70        80        90        100
                            ....|....|....|....|....|....|....|....|....|....|
                SEC5        SEVFRYSLQKLAMTVSRTGRQVLGERRQRAPN-----------------
                gi|4885521| SEVFRYSLQKLAMTVSRTGRQVLGERRQRAPN-----------------
                gi|6754864| SEVFRYSLQKLAHTVSRTGRQVLGERRQRAPN-----------------
                gi|16758386|SEVFRYSLQKLAHTVSRTGRQVLGERRHRAPN-----------------
                gi|12833393|SEVFRYSLQKLAHTVSRTGRQVLGERSSEPPTEAPAPSPGRPCHQVLLCF
                gi|1083432| --VFRYSLQKLAHTVSRTGRQVLGERRQRAPN-----------------

                                  110       120       130       140       150
                            ....|....|....|....|....|....|....|....|....|....|
                SEC5        --------------------------------------------------
                gi|4885521| --------------------------------------------------
                gi|6754864| --------------------------------------------------
                gi|16758386|--------------------------------------------------
                gi|12833393|STAWEPVPRRNGGSLCLLVRVALAKVSEGPVAPRKAAPTMMKISVPFPAP
                gi|1083432| --------------------------------------------------

                                  160       170       180       190       200
                            ....|....|....|....|....|....|....|....|....|....|
                SEC5        --------------------------------------------------
                gi|4885521| --------------------------------------------------
                gi|6754864| --------------------------------------------------
                gi|16758386|--------------------------------------------------
                gi|12833393|LPLSHYRRVGEEGGRGEQPSRYGRRHHILIWTKSEQYHLSRTRSYHEDRT
                gi|1083432| --------------------------------------------------

                            ....|....
                SEC5        ---------
                gi|4885521| ---------
                gi|6754864| ---------
                gi|16758386|---------
                gi|12833393|AHQPAEWTF
                gi|1083432| ---------
```

Neuronatin, disclosed herein as SEC5, is a brain-specific human gene isolated and observed to be selectively expressed during brain development. The human gene spans 3973 bases and contains three exons and two introns. Based on primer extension analysis, a single

5   cap site is located 124 bases upstream from the methionine (ATG) initiation codon, in good context, GAACCATGG. The promoter contains a modified TATA box, CATAAA (-27), and a modified CAAT box, GGCGAAT (-59). The 5'-flanking region contains putative transcription factor binding sites for SP-1, AP-2 (two sites), delta-subunit, SRE-2, NF-A1, and ETS. In addition, a 21-base sequence highly homologous to the neural restrictive silence

10   element that governs neuron-specific gene expression is observed at -421. Furthermore, SP-1 and AP-3 binding sites are present in intron 1. All splice donor and acceptor sites conformed

to the GT/AG rule. Exon 1 encodes 24 amino acids, exon 2 encodes 27 amino acids, and exon 3 encodes 30 amino acids. At the 3'-end of the gene, the poly(A) signal, AATAAA, poly(A) site, and GT cluster are observed. The neuronatin gene is expressed as two mRNA species, alpha and beta, generated by alternative splicing. The alpha-form contains all three exons,

5   whereas in the beta-form, the middle exon has been spliced out. The third nucleotide of all frequently used codons, except threonine, of neuronatin is either G or C, consistent with codon usage expected for Homo sapiens.

The human neuronatin gene on chromosome 20q11.2 is imprinted and transcribed specifically from the paternal allele. The region containing neuronatin has multiple CpG

10   islands, and methylation analysis showed that a 1.8-kb CpG island in its promoter region exhibits differential methylation in all tissues examined. Neuronatin lies within the singular 8.5-kb intron of the gene encoding bladder cancer-associated protein (BLCAP). Northern blot analysis reveals that the human neuronatin message is expressed predominantly in the fetal brain in the brain-specific manner, but only faintly in the adult brain. Strong neuronatin

15   expression is observed in the anterior pituitary gland, and in several human pituitary adenomas, including ACTH-producing, GH-producing, and nonfunctioning adenomas.

The SEC5 disclosed in this invention is expressed in at least the following tissues: apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus,

20   CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, those that express MHC I, II, and III, nervous tissues, medulla, subthalamic nucleus, ovary,

25   pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortia) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, public EST sources, literature sources, and/or RACE sources.

30   Further expression data for SEC5 is provided in Example 2.

The nucleic acids and proteins of SEC5 are useful in potential therapeutic applications implicated in various SEC-related pathological disorders described further herein. The SEC5 nucleic acid encoding the neuronatin-like protein of the invention, or fragments thereof, may

35

further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

These materials are further useful in the generation of antibodies that bind immunospecifically to the substances of the invention for use in therapeutic or diagnostic

5   methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-SECX Antibodies" section below. The disclosed SEC5 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, for example, a contemplated SEC5 epitope comprises from about amino acids 33 to about 38. In another embodiment, for example, a

10   SEC5 epitope comprises from about amino acids 40 to about 81.

SEC6

The disclosed SEC6 (alternatively referred to herein as CG56157-01) includes the 679 nucleotide sequence (SEQ ID NO:11) shown in Table 6A. A SEC6 ORF begins with a Kozak

15   consensus ATG initiation codon at nucleotides 70-72 and ends with a TAA codon at nucleotides 665-667.

Table 6A. SEC6 Nucleotide Sequence (SEQ ID NO:11)
GGCGGCGTTCGTGTCCGAGGTCACTAGTTTCCCGGTAGTTCAGCTGCACATGAATAGAAC AGCAATGAGAGCCAGTCAGAAGGACTTTGAAAATTCAATAAATCAAGTGAAACTCTTGAA AAAGGATCCAGGAAACGAAGTGAAGCTAAAACTCTACGCGCTATATAAGCAGGCCACTGA AGGACCTTGTAACATGCCCAAACCAGGTGTATTTGACTTGATCAACAAGGCCAAATGGGA CGCATGGAATGCCCTTGGCAGCCTGCCCAAGGAAGCTGCCAGGCAGAACTATGTGGATTT GGTGTCCAGTTTGAGTCCTTCATTGGAATCCTCTAGTCAGGTGGAGCCTGGAACAGACAG GAAATCAACTGGGTTTGAAACTCTGGTGGTGACCTCCGAAGATGGCATCACAAAGATCAT GTTCAACCGGCCCAAAAAGAAAAATGCCATAAACACTGAGATGTATCATGAAATTATGCG TGCACTTAAAGCTGCCAGCAAGGATGACTCAATCATCACTGTTTTAACAGGAAATGGTGA CTATTACAGTAGTGGGAATGATCTGACTAACTTCACTGATATTCCCCCTGGTGGAGTANA GGAGAAAGCTAAAAATAATGCCGTTTTACTGAAGGGAATTTGTGGGCTGTTTTATAGAAT TTCCTAAGCCTCTGATTGC

The SEC6 polypeptide (SEQ ID NO:12) encoded by SEQ ID NO:11 is 205 amino

20   acids in length and is presented using the one-letter amino acid code in Table 6B. The Psort profile for SEC6 predicts that this sequence is likely to be localized at the nucleus with a certainty of 0.6000. In alternative embodiments, a SEC6 polypeptide is located to lysosomes with a certainty of 0.1000, or to the mitochondrial matrix space with a certainty of 0.3600.

Table 6B. SEC6 protein sequence (SEQ ID NO:12)

```
MNRTAMRASQKDFENSINQVKLLKKDPGNEVKLKLYALYKQATEGPCNMPKPGVFDLINK
AKWDAWNALGSLPKEAARQNYVDLVSSLSPSLESSSQVEPGTDRKSTGFETLVVTSEDGI
TKIMFNRPKKKNAINTEMYHEIMRALKAASKDDSIITVLTGNGDYYSSGNDLTNFTDIPP
GGVXEKAKNNAVLLKGICGLFYRIS
```

A BLAST analysis of SEC6 was run against the proprietary PatP GENESEQ Protein
Patent database. It was found, for example, that the amino acid sequence of SEC6 had high
homology to other proteins as shown in Table 6C.

5

Table 6C. BLASTX results from PatP database for SEC6		
Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum Probability P(N)
patp:AAY07017 Breast cancer associated antigen precursor	1044	2.9e-105
patp:AAM93539 Human polypeptide	999	1.7e-100
patp:AAB81822 Human endozepine-like ENDO9	974	7.6e-98
patp:AAB63531 Human gastric cancer associated antigen	956	6.1e-96
patp:AAB63535 Human gastric cancer associated antigen	947	5.5e-95

In a search of public sequence databases, it was found, for example, that the amino
acid sequence of the SEC6 protein of the present invention was found to have 193 of 210
amino acid residues (96%) identical to the 394 amino acid residue ptnr:SPTREMBL-ACC:Q9QW37

10   protein from Rattus sp (rat) (OR18 odorant receptor, E=4e-92). SEC6 also has homology to
the proteins shown in the BLASTP data in Table 6D.

Table 6D. SEC6 BLASTP results					
Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positive (%)	Expect
gi|12052810|e mb|CAB66577.1 | (AL136642)	hypothetical protein [Homo sapiens]	394	193/201 (96)	195/201 (96)	e-109
gi|12803665|g b|AAH02668.1| AAH02668 (BC002668)	peroxisomal D3,D2-enoyl-CoA isomerase [Homo sapiens]	394	194/201 (96)	196/201 (96)	e-102
gi|8574030|em b|CAB94781.1| (AL033383)	dJ1013A10.3 (related to DBI (diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein)) [Homo sapiens]	374	194/201 (96)	196/201 (96)	e-101
gi|7670842|gb |AAF66247.1|A F244138 1 (AF244138)	hepatocellular carcinoma-associated antigen 88 [Homo sapiens]	364	194/201 (96)	196/201 (96)	e-101
gi|3193336|gb |AAC19317.1| (AF069301)	DBI-related protein [Homo sapiens]	364	193/201 (96)	195/201 (96)	e-100

This BLASTP data is displayed graphically in the ClustalW in Table 6E. A multiple sequence alignment is given in Table 6E, with the SEC6 protein being shown on line 1, in a ClustalW analysis comparing the protein of the invention with the related protein sequences shown in Table 6D.

```
Table 6E. ClustalW Alignment of SEC6

   1)SEC6    (SEQ ID NO:12)
   2)gi|12052810|  (SEQ ID NO:66)
   3)gi|12803665|  (SEQ ID NO:67)
   4)gi|8574030|   (SEQ ID NO:68)
   5)gi|7672423|   (SEQ ID NO:69)
   6)gi|3193336|   (SEQ ID NO:70)
                        10        20        30        40        50
                   ....|....|....|....|....|....|....|....|....|....|
   SEC6            -----------------------------MNRTAMRASQKDFENSMNQV
   gi|12052810|    MAMAYLAWRLARRSCPSSLQVTSFPVVQLHMNRTAMRASQKDFENSMNQV
   gi|12803665|    MAMAYLAWRLARRSCPSSLQVTSFPVVQLHMNRTAMRASQKDFENSMNQV
   gi|8574030|     ------------------VTSFPVVQLHMNRTAMRASQKDFENSMNQV
   gi|7672423|     --------------------MLLSIIVALCLWLRIALGVRGAPCEAVRI
   gi|3193336|     -----------------------------MNRTAMRASQKDFENSMNQV

                        60        70        80        90       100
                   ....|....|....|....|....|....|....|....|....|....|
   SEC6            KLLKKDPGNEVKLKLYALYKQATEGPCNMPKPGVFDLINKAKWDAWNALG
   gi|12052810|    KLLKKDPGNEVKLKLYALYKQATEGPCNMPKPGVFDLINKAKWDAWNALG
   gi|12803665|    KLLKKDPGNEVKLKLYALYKQATEGPCNMPKPGVFDLINKAKWDAWNALG
   gi|8574030|     KLLKKDPGNEVKLKLYALYKQATEGPCNMPKPGVFDLINKAKWDAWNALG
   gi|7672423|     PMCRHMPWNITRMPNHLHHSTQEN---AILAICQYEEIVDVNCSSVLSFF
   gi|3193336|     KLLKKDPGNEVKLKLYALYKQATEGPCNMPKPGVFDLINKAKWDAWNALG

                       110       120       130       140       150
                   ....|....|....|....|....|....|....|....|....|....|
   SEC6            SLPKEAARQNYVDLVSSLSPSLESSSQVEPGTDRKSTGFETLVVTSEDGI
   gi|12052810|    SLPKEAARQNYVDLVSSLCPSLESSSQVEPGTDRKSTGFETLVVTSEDGI
   gi|12803665|    SLPKEAARQNYVDLVSSLSPSLESSSQVEPGTDRKSTGFETLVVTSEDGI
   gi|8574030|     SLPKEAARQNYVDLVSSLSPSLESSSQVEPGTDRKSTGFETLVVTSEDGI
   gi|7672423|     LCAMYAPICTLEFLHDPIKPCKSVCQRARDDCIPLMKMYNHSWPESLACD
   gi|3193336|     SLPKEAARQNYVDLVSSLSPSLESSSQVEPGTDRKSTGFETLVVTSEDGI

                       160       170       180       190       200
                   ....|....|....|....|....|....|....|....|....|....|
   SEC6            TKIMFNR--PKKKNAINTEMYHEIMRALKAASKDDSIITVLTGNGDYYSS
   gi|12052810|    TKIMFNR--PKKKNAINTEMYHEIMRALKAASKDDSIITVLTGNGDYYSS
   gi|12803665|    TKIMFNR--PKKKNAINTEMYHEIMRALKAASKDDSIITVLTGNGDYYSS
   gi|8574030|     TKIMFNR--PKKKNAINTEMYHEIMRALKAASKDDSIITVLTGNGDYYSS
   gi|7672423|     ELPVYDRGVCISPEAIVTDIPEDVKWIDITP---DMMVQERSFDADCKHL
   gi|3193336|     TKIMFNR--PKKKNAINTEMYHEIMRALKAASKDDSIITVLTGNGDCYSS

                       210       220       230       240       250
                   ....|....|....|....|....|....|....|....|....|....|
   SEC6            GNDLTNFTDIPPGGVXEKAKNNAVLLKGICGLFYRIS-------------
   gi|12052810|    GNDLTNFTDIPPGGVEEKAKNNAVLLREFVGCFIDFPKPLIAVVNGPAVG
   gi|12803665|    GNDLTNFTDIPPGGVEEKAKNNAVLLREFVGCFIDFPKPLIAVVNGPAVG
   gi|8574030|     GNDLTNFTDIPPGGVEEKAKNNAVLLREFVGCFIDFPKPLIAVVNGPAVG
   gi|7672423|     SPDRCKCKKVKPTLATYLSKNYSYVIHAKIKAVQRS--------GCNEVT
   gi|3193336|     GNDLTNFTDIPPGGVEEKAKNNAVLLREFVGCFIDFPKPLIAVVNGPAVG

                       260       270       280       290       300
                   ....|....|....|....|....|....|....|....|....|....|
   SEC6            --------------------------------------------------
```

```
gi|12052810|    ISVTLLGLFDAVYASDRATFHTPFSHLGQSPEGCSSYTFPKIMSPAKATE
gi|12803665|    ISVTLLGLFDAVYASDRATFHTPFSHLGQSPEGCSSYTFPKIMSPAKATE
gi|8574030|     ISVTLLGLFDAVYASDRATFHTPFSHLGQSPEGCSSYTFPKIMSPAKATE
gi|7672423|     TVVDVKEIFKSSSPIPRTQVPLITNSSCQCPHILPHQDVLIMCYERRSRM
gi|3193336|     ISVTLLGLFDAVYASDRATFHTPFSHLGQSPEGCSSYTFPKIMSPAKATE

                    310       320       330       340       350
                ....|....|....|....|....|....|....|....|....|....|
SEC6            --------------------------------------------------
gi|12052810|    MLIFGKKLTAGEACAQGLVTEVFPDSTFQKEVWTRLKAFAKLPPNALRIS
gi|12803665|    MLIFGKKLTAGEACAQGLVTEVFPDSTFQKEVWTRLKAFAKLPPNALRIS
gi|8574030|     MLIFGKKLTAGEACAQGLVTEVFPDSTFQKEVWTRLKAFAKLPPNALRIS
gi|7672423|     MLIENCLVEK----WRDQIS--RRSIQWEERLQEQQRTTQDKKQIASRIS
gi|3193336|     MLIFGKKLTAGEACAQGLVTEVFPDSTFQKEVWTRLKAFAKLPPNVLRIS

                    360       370       380       390
                ....|....|....|....|....|....|....|....|....|.
SEC6            ----------------------------------------------
gi|12052810|    KEVIRKREREKLHAVNAEECNVLQGRWLSDECTNAVVNFLSRKSKL
gi|12803665|    KEVIRKREREKLHAVNAEECNVLQGRWLSDECTNAVVNFLSRKSKL
gi|8574030|     KEVIRKREREKLHAVNAEECNVLQGRWLSDECTNAVVNFLSRKSKL
gi|7672423|     RSNPPKPKGRSPASKPASPKKNIKARSAPKKSN------PKKSTS
gi|3193336|     KEVIRKREREKLHAVNAEECNVLQGRWLSDECTNAVVNFLSRKSKL
```

The presence of identifiable domains in SEC6, as well as all other SECX proteins, was determined by searches using software algorithms such as PROSITE, DOMAIN, Blocks, Pfam, ProDomain, and Prints, and then determining the Interpro number by crossing the

5   domain match (or numbers) using the Interpro website (http:www.ebi.ac.uk/ interpro). DOMAIN results for SEC6 as disclosed in Table 6F, were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST analyses. This BLAST analysis software samples domains found in the Smart and Pfam collections. Fully conserved single residues are indicated by the sign (|) and "strong" semi-conserved residues are indicated

10   by the sign (+). The "strong" group of conserved amino acid residues may be any one of the following groups of amino acids: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

Table 6F lists the domain description from DOMAIN analysis results against SEC6. This indicates that the SEC6 sequence has properties similar to those of other proteins known

15   to contain this domain.

Table 6F. Domain Analysis of SEC6
gnl|Pfam|pfam00887, ACBP, Acyl CoA binding protein.
CD-Length = 85 residues, 89.4% aligned
Score = 101 bits (252), Expect = 4-23e

```
SEC 6:    9   SQKDFENSINQVKLLKKDPGNEVKLKLYALYKQATEGPCNMPKPGVFDLINKAKWDAWNA  68
              Q+ FE +  +VK LKK+P N+  L+LY+LYKQAT G CN  KPG+FDL +AKWDAWN
Sbjct:    1   LQEQFEAAAEKVKKLKKNPSNDELLQLYSLYKQATVGDCNTEKPGMFDLKGRAKWDAWNE  60
```

```
SEC 6:   69   LGSLPKEAARQNYVDL   84
              L   + KE A + Y+
Sbjct:   61   LKGMSKEEAMKAYIAK   76 (SEQ ID NO: 300)
```

Mechanisms for formation of drug dependence and expression of withdrawal
syndrome have not fully clarified despite of huge accumulation of experimental and clinical
data at present. Several clinical features of withdrawal syndrome are considered to be common
among patients with drug dependence induced by different drugs of abuse. One of them is
anxiety. Recent investigations have revealed that diazepam binding inhibitor (DBI), disclosed
herein as SEC6 serves as an inverse agonist for benzodiazepine (BZD) receptors with
endogenously anxiogenic potential. Cerebral DBI expression in brain participates in the
formation of drug dependence and/or emergence of withdrawal syndrome. Cerebral DBI
expression significantly increases in mammals with drug dependence induced by drugs such
as, for example, morphine, nicotine, and alcohol. In the cases of nicotine- and morphine-
dependent mice concomitant administration of antagonists for nicotinic acetylcholine and
opioid receptors, respectively, abolished the increase. Abrupt cessation of administration of
drugs facilitated further increase in DBI expression. Therefore, these alterations in DBI
expression have close relationship with formation of drug dependence and/or emergence of
withdrawal syndrome, and are considered to be a common biochemical process in drug
dependence induced by different drugs of abuse. DBI, and the modulation of DBI expression
and activity thus provides for therapeutic targets for common biochemical pathways involved
in drug dependence and provides a method of preventing the formation of drug dependence
and/or the emergence of withdrawal syndromes.

The SEC6 disclosed in this invention is expressed in at least the following tissues:
apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain,
Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus,
CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells,
endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye,
frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver,
fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, those
that express MHC I, II, and III, nervous tissues, medulla, subthalamic nucleus, ovary,
pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine,
smooth muscle (coronary artery in aortia) spinal cord, spleen, stomach, taste receptor cells of
the tongue, testis, thalamus, and thymus tissue. This information was derived by determining
the tissue sources of the sequences that were included in the invention including but not

limited to SeqCalling sources, public EST sources, literature sources, and/or RACE sources. Further expression data for SEC6 is provided in Example 2.

The nucleic acids and proteins of SEC6 are useful in potential therapeutic applications implicated in various SEC-related pathological disorders described further herein. The SEC6

5   nucleic acid encoding the DBI-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

These materials are further useful in the generation of antibodies that bind immunospecifically to the substances of the invention for use in therapeutic or diagnostic

10   methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-SECX Antibodies" section below. The disclosed SEC6 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, for example, a contemplated SEC6 epitope comprises from about amino acids 1 to about 93. In another embodiment, for example, a

15   SEC6 epitope comprises from about amino acids 95 to about 115. In further embodiments, for example, a SEC6 epitope comprises from about 120 to about 160, and from about 165 to about 195.

SEC7

20   The disclosed SEC7 (alternatively referred to herein as CG56159-01) includes the 3046 nucleotide sequence (SEQ ID NO:13) shown in Table 7A. A SEC7 ORF begins with a Kozak consensus ATG initiation codon at nucleotides 61-63 and ends with a TAG codon at nucleotides 2960-2962.

Table 7A. SEC7 Nucleotide Sequence (SEQ ID NO:13)
TGGGGCTGCTCCGTTCTCTGCCTGGCCTGAGGCTCCCTGAGCCGCCTCCCCACCATCACC ATGGCCAAGGGCTTCTATATTTCCAAGTCCCTGGGCATCCTGGGGATCCTCCTGGGCGTG GCAGCCGTGTGCACAATCATCGCACTGTCAGTGGTGTACTCCCAGGAGAAGAACAAGAAC GCCAACAGCTCCCCCGTGGCCTCCACCACCCCGTCCGCCTCAGCCACCACCAACCCCGCC TCGGCCACCACCTTGGACCAAAGTAAAGCGTGGAATCGTTACCGCCTCCCCAACACGCTG AAACCCGATTCCTACCAGGTGACGCTGAGACCGTACCTCACCCCCAATGACAGGGGCCTG TACGTTTTTAAGGGCTCCAGCACCGTCCGTTTCACCTGCAAGGAGGCCACTGACGTCATC ATCATCCACAGCAAGAAGCTCAACTACACCCTCAGCCAGGGGCACAGGGTGGTCCTGCGT GGTGTGGGGAGGCTCCCAGCCCCCCGACATTGACAAGACTGAGCTGGTGGAGCCCACCGAG TACCTGGTGGTGCACCTCAAGGGCTCCCTGGTGAAGGACAGCCAGTATGAGATGGACAGC GAGTTCGAGGGGGAGTTGGCAGATGACCTGGCGGGCTTCTACCGCAGCGAGTACATGGAG GGCAATGTCAGAAAGGTGGTGGCCACTACACAGATGCAGGCTGCAGATGCCCGGAAGTCC TTCCCATGCTTCGATGAGCCGGCCATGAAGGCCGAGTTCAACATCACGCTTATCCACCCC AAGGACCTGACAGCCCTGTCCAACATGCTTCCCAAAGGTCCCAGCACCCCACTTCCAGAA GACCCCAACTGGAATGTCACTGAGTTCCACACCACGCCCAAGATGTCCACGTACTTGCTG GCCTTCATTGTCAGTGAGTTCGACTACGTGGAGAAGCAGGCATCCAATGGTGTCTTGATC

```
CGGATCTGGGCCCGGCCCAGTGCCATTGCGGCGGGCCACGGCGATTATGCCCTGAACGTG
ACGGGCCCCATCCTTAACTTCTTTGCTGGTCATTATGACACACCCTACCCACTCCCAAAA
TCAGACCAGATTGGCCTGCCAGACTTCAACGCCGGCGCCATGGAGAACTGGGGACTGGTG
ACCTACCGGGAGAACTCCCTGCTGTTCGACCCCCTGTCCTCCTCCAGCAGCAACAAGGAG
CGGGTGGTCACTGTGATTGCTCATGAGCTGGCCCACCAGTGGTTCGGGAACCTGGTGACC
ATAGAGTGGTGGAATGACCTGTGGCTGAACGAGGGCTTCGCCTCCTACGTGGAGTACCTG
GGTGCTGACTATGCGGAGCCCACCTGGAACTTGAAAGACCTCATGGTGCTGAATGATGTG
TACCGCGTGATGGCAGTGGATGCACTGGCCTCCTCCCACCCGCTGTCCACACCCGCCTCG
GAGATCAACACGCCGGCCCAGATCAGTGAGCTGTTTGACGCCATCTCCTACGACAAGGGC
GCCTCAGTCCTCAGGATGCTCTCCAGCTTCCTGTCCGAGGACGTATTCAAGCAGGGCCTG
GCGTCCTACCTCCACACCTTTGCCTACCAGAACACCATCTACCTGAACCTGTGGGACCAC
CTGCAGGAGGCTGTGAACAACCGGTCCATCCAACTCCCCACCACCGAGCGGGACATCATG
AACCGCTGGACCCTGCAGATGGGCTTCCCGGTCATCACGGTGGATACCAGCACGGGGACC
CTTTCCCAGGAGCACTTCCTCCTTGACCCCGATTCCAATGTTACCCGCCCCTCAGAATTC
AACTACGTGTGGATTGTGCCCATCACATCCATCAGAGATGGCAGACAGCAGCAGGACTAC
TGGCTGATGGATGTAAGAGCCCAGAACGATCTCTTCAGCACATCAGGCAATGAGTGGGTC
CTGCTGAACCTCAATGTGACGGGCTATTACCGGGTGAACTACGACGAAGAGAACTGGAGG
AAGATTCAGACTCAGCTGCAGAGAGACCACTCGGCCATCCCTGTCATCAATCGGGCACAG
ATCATTAATGACGCCTTCAACCTGGCCAGTGCCCATAAGGTCCCTGTCACTCTGGCGCTG
AACAACACCCTCTTCCTGATTGAAGAGAGACAGTACATGCCCTGGGAGGCCGCCCTGAGC
AGCCTGAGCTACTTCAAGCTCATGTTTGACCGCTCCGAGGTCTATGGCCCCATGAAGAAC
TACCTGAAGAAGCAGGTCACACCCCTCTTCATTCACTTCAGAAATAATACCAACAACTGG
AGGGAGATCCCAGAAAACCTGATGGACCAGTACAGCGAGGTTAATGCCATCAGCACCGCC
TGCTCCAACGGAGTTCCAGAGTGTGAGGAGATGGTCTCTGGCCTTTTCAAGCAGTGGATG
GAGAACCCCAATAATAACCCGATCCACCCCAACCTGCGGTCCACCGTCTACTGCAACGCT
ATCGCCCAGGGCGGGGAGGAGGAGTGGGACTTCGCCTGGGAGCAGTTCCGAAATGCCACA
CTGGTCAATGAGGCTGACAAGCTCCGGGCAGCCCTGGCCTGCAGCAAAGAGTTGTGGATC
CTGAACAGGTACCTGAGCTACACCCTGAACCCGGACTTAATCCGGAAGCAGGACGCCACC
TCTACCATCATCAGCATTACCAACAACGTCATTGGGCAAGGTCTGGTCTGGGACTTTGTC
CAGAGCAACTGGAAGAAGCCTTTTAACGATTATGGTGGTGGCTCGTTCTCCTTCTCCAAC
CTCATCCAGGCAGTGACACGACGATTCTCCACCGAGTATGAGCTGCAGCAGCTGGAGCAG
TTCAAGAAGGACAACGAGGAAACAGGCTTCGGCTCAGGCACCCGGGCCCTGGAGCAAGCC
CTGGAGAAGACGAAAGCCAACATCAAGTGGGTGAAGGAGAACAAGGAGGTGGTGCTCCAG
TGGTTCACAGAAAACAGCAAATAGTCCCCAGCCCTTGAAGCTACCCGGCCCCGATCGAAG
GTGCCCACATGTGTCCATCCCAGCGGCTGGTGCAGGGCCTCCATTC
```

The SEC7 polypeptide (SEQ ID NO:14) encoded by SEQ ID NO:13 is 967 amino acids in length and is presented using the one-letter amino acid code in Table 7B. The Psort profile for SEC7 predicts that this sequence has a signal peptide and is likely to be secreted from the cell with a certainty of 0.8200. In alternative embodiments, a SEC7 polypeptide is located to lysosomes with a certainty of 0.1900, to the endoplasmic reticulum with a certainty of 0.1000. The Signal P predicts a likely cleavage site for a SEC7 peptide is between positions 34 and 35, *i.e.*, at the dash in the sequence VYS-QE.

<div style="border:1px solid">

Table 7B. SEC7 protein sequence (SEQ ID NO:14)

```
MAKGFYISKSLGILGILLGVAAVCTIIALSVVYSQEKNKNANSSPVASTTPSASATTNPA
SATTLDQSKAWNRYRLPNTLKPDSYQVTLRPYLTPNDRGLYVFKGSSTVRFTCKEATDVI
IIHSKKLNYTLSQGHRVVLRGVGGSQPPDIDKTELVEPTEYLVVHLKGSLVKDSQYEMDS
EFEGELADDLAGFYRSEYMEGNVRKVVATTQMQAADARKSFPCFDEPAMKAEFNITLIHP
KDLTALSNMLPKGPSTPLPEDPNWNVTEFHTTPKMSTYLLAFIVSEFDYVEKQASNGVLI
RIWARPSAIAAGHGDYALNVTGPILNFFAGHYDTPYPLPKSDQIGLPDFNAGAMENWGLV
TYRENSLLFDPLSSSSSNKERVVTVIAHELAHQWFGNLVTIEWWNDLWLNEGFASYVEYL
GADYAEPTWNLKDLMVLNDVYRVMAVDALASSHPLSTPASEINTPAQISELFDAISYSKG
ASVLRMLSSFLSEDVFKQGLASYLHTFAYQNTIYLNLWDHLQEAVNNRSIQLPTTERDIM
NRWTLQMGFPVITVDTSTGTLSQEHFLLDPDSNVTRPSEFNYVWIVPITSIRDGRQQQDY
WLMDVRAQNDLFSTSGNEWVLLNLNVTGYYRVNYDEENWRKIQTQLQRDHSAIPVINRAQ
IINDAFNLASAHKVPVTLALNNTLFLIEERQYMPWEAALSSLSYFKLMFDRSEVYGPMKN
```

</div>

```
YLKKQVTPLFIHFRNNTNNWREIPENLMDQYSEVNAISTACSNGVPECEEMVSGLFKQWM
ENPNNNPIHPNLRSTVYCNAIAQGGEEEWDFAWEQFRNATLVNEADKLRAALACSKELWI
LNRYLSYTLNPDLIRKQDATSTIISITNNVIGQGLVWDFVQSNWKKPFNDYGGGSFSFSN
LIQAVTRRFSTEYELQQLEQFKKDNEETGFGSGTRALEQALEKTKANIKWVKENKEVVLQ
WFTENSK
```

A BLAST analysis of SEC7 was run against the proprietary PatP GENESEQ Protein Patent database. It was found, for example, that the amino acid sequence of SEC7 had high homology to other proteins as shown in Table 7C.

Table 7C. BLASTX results from PatP database for SEC7

```
                                                         Smallest
                                                           Sum
                                                High     Probability
Sequences producing High-scoring Segment Pairs: Score    P(N)

patp:AAW93621 Human CD13/aminopeptidase N protein  5066   0.0
patp:AAB54345 Human pancreatic cancer antigen protein 5059 0.0
patp:AAU12270 Human PRO5995 polypeptide sequence   1486   4.2e-152
patp:AAB24422 Human PRO1154 protein                1279   3.6e-130
patp:AAY66736 Membrane-bound protein PRO1154       1279   3.6e-130
```

In a search of public sequence databases, it was found, for example, that the amino acid sequence of the SEC7 protein of the present invention was found to have 734 of 967 amino acid residues (75%) identical to the 996 amino acid NM_008486. SEC7 also has homology to the other proteins shown in the BLASTP data in Table 7D.

Table 7D. SEC7 BLASTP results

Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positive (%)	Expect
gi|6678664|ref|NP_032512.1| (NM_008486)	leucine arylaminopeptidase 1, intestinal; aminopeptidase M; aminopeptidase N; microsomal aminopeptidase [Mus musculus]	966	734/967 (75)	841/967 (86)	0.0
gi|1351929|sp|P15541|AMPN_RABIT	AMINOPEPTIDASE N (MICROSOMAL AMINOPEPTIDASE) (LEUKEMIA ANTIGEN CD13)	966	772/970 (79)	869/970 (89)	0.0
gi|16877511|gb|AAH17011.1| AAH17011 (BC017011)	Similar to alanyl (membrane) aminopeptidase [Mus musculus]	974	738/975 (75)	840/975 (85)	0.0

| gi\|4502095\|ref\|NP_001141.1_ (NM_001150) | membrane alanine aminopeptidase precursor; microsomal aminopeptidase; Alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, [Homo sapiens] | 967 | 964/967 (99) | 965/967 (99) | 0.0 |
| gi\|113743\|sp\|P15144\|AMPN_HUMAN | AMINOPEPTIDASE N (MICROSOMAL AMINOPEPTIDASE) (GP150) (MYELOID PLASMA MEMBRANE GLYCOPROTEIN CD13) | 967 | 967/967 (100) | 967/967 (100) | 0.0 |

This BLASTP data is displayed graphically in the ClustalW in Table 7E. A multiple sequence alignment is given in Table 7E, with the SEC7 protein being shown on line 1, in a ClustalW analysis comparing the protein of the invention with the related protein sequences

5   shown in Table 7D.

Table 7E. ClustalW Alignment of SEC7

```
1)SEC7   (SEQ ID NO:14)
2)gi|6678664|  (SEQ ID NO:71)
3)gi|1351929|  (SEQ ID NO:72)
4)gi|16877511| (SEQ ID NO:73)
5)gi|4502095|  (SEQ ID NO:74)
6)gi|113743|   (SEQ ID NO:75)
                    10        20        30        40        50
              ....|....|....|....|....|....|....|....|....|....|
SEC7          MAKGFYISKSLGILGILLGVAAVCTIIALSVVYSQEKNKNANSSPVASTT
gi|6678664|   MAKGFYISKSLGILGILLGVAAVCTIIALSVVYAQEKNRNAENSATAPTL
gi|1351929|   MAKGFYISKSLGILGILLGVAAVCTIMALSVVYRQEKNKNTSQSPSMAPL
gi|16877511|  MAKGFYISKSLGILGILLGVAAVCTIIALSVVYAQEKNRNAENSATAPTL
gi|4502095|   MAKGFYISKSLGILGILLGVAAVCTIIALSVVYSQEKNKNANSSPVASTT
gi|113743|    MAKGFYISKSLGILGILLGVAAVCTIIALSVVYSQEKNKNANSSPVASTT

                    60        70        80        90        100
              ....|....|....|....|....|....|....|....|....|....|
SEC7          PSASATTNPAS-------ATTLDQSKAWNRYRLPNTLKPDSYQVTLRPY
gi|6678664|   PGSTSATTATE-------TPAVDESKPWNQYRLPKTLIPDAYRVILRPY
gi|1351929|   N-PTATSSP--------ATTLDQNLPWNRYRLPKTLIPDSYNVVLRPY
gi|16877511|  PGSTSATTATETATTTATTTPAVDESKPWNQYRLPKTLIPDSYRVILRPY
gi|4502095|   PSASATTNPAS-------ATTLDQSKAWNRYRLPNTLKPDSYQVTLRPY
gi|113743|    PSASATTNPAS-------ATTLDQSKAWNRYRLPNTLKPDSYQVTLRPY

                    110       120       130       140       150
              ....|....|....|....|....|....|....|....|....|....|
SEC7          LTPNDRGLYVFKGSSTVRFTCKEATDVIIHSKKLNYTLSQGHRVVLRGV
gi|6678664|   LTPNNQGLYIFQCNSTVRFTCNQTTDVIIHSKKLNYTLKGNHRVVLRTL
gi|1351929|   LSPNSQGLYIFTGSSTVRFTCQEATNVIIHSKKLNYTITQGHRVVLRTL
gi|16877511|  LTPNNQGLYIFQGSSTVRFTCNQTDVIIHSKKLNYTLKGNHRVVLRTL
gi|4502095|   LTPNDRGLYIFKGSSTVRFTCKEATDVIIHSKKLNYTLSQGHRVVLRGV
gi|113743|    LTPNDRGLYVFKGSSTVRFTCKEATDVIIHSKKLNYTLSQGHRVVLRGV

                    160       170       180       190       200
              ....|....|....|....|....|....|....|....|....|....|
SEC7          GGSQPPDIDKTELVEPTEYLVVHLKGSLVKDSQYEMDSEFEGELADDLAG
gi|6678664|   DGTPAPNIDKTELVERTEYLVVHLKGSLVEGRQYEMDSQFQGELADDLAG
gi|1351929|   RGSQPPAIASTELVELTEYLVVHLQGQLVAGSQYEMDTKFQGELADDLAG
gi|16877511|  DGTPAPNIDKTELVERTEYLVVHLQGSLVEGRQYEMDSEFQGELADDLAG
gi|4502095|   GGSQPPDIDKTELVEPTEYLVVHLKGSLVKDSQYEMDSEFEGELADDLAG
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gi|113743|        GGSQPPDIDKTELVEPTEYLVVHLKGSLVKDSQYEMDSEFEGELADDLAG

                    210       220       230       240       250
                 ....|....|....|....|....|....|....|....|....|....|
SEC7             FYRSEYMEGNVRKVVATTQMQAADARKSFPCFDEPAMKAEFNITLIHPKD
gi|6678664|      FYRSEYMEGDVKKVVATTQMQAADARKSFPCFDEPAMKAMFNITLIMPNN
gi|1351929|      FYRSEYMEGNVRKVVATTQMQAADARKSFPCFDEPAMKATFNITPIHPRD
gi|16877511|     FYRSEYMEGGVKKVVATTQMQAADARKSFPCFDEPAMKAMFNITLIMPNN
gi|4502095|      FYRSEYMEGNVRKVVATTQMQAADARKSFPCFDEPAMKAEFNITLIHPKD
gi|113743|       FYRSEYMEGNVRKVVATTQMQAADARKSFPCFDEPAMKAEFNITLIHPKD

                    260       270       280       290       300
                 ....|....|....|....|....|....|....|....|....|....|
SEC7             LTALSNMLPKGPSTPLPEDPNWNVTEFHTTPKMSTYLLAEIVSEFDYVEK
gi|6678664|      LTALSNMLPK-ESKPYPEDPSCTMTEFHSTPKMSTYLLAYIVSEFKNISS
gi|1351929|      YTALSNMLPR-SSTALPEDPNWTVTEFHTTPKMSTYLLAYIVSEFTNIEA
gi|16877511|     LTALSNMLPK-ESKPYPEDPSCTMTEFHSTPKMSTYLLAYIVSEFKNISS
gi|4502095|      LTALSNMLPKGPSTPLPEDPNWNVTEFHTTPKMSTYLLAEIVSEFDYVEK
gi|113743|       LTALSNMLPKGPSTPLPEDPNWNVTEFHTTPKMSTYLLAEIVSEFDYVEK

                    310       320       330       340       350
                 ....|....|....|....|....|....|....|....|....|....|
SEC7             QASNGVLIRIWARPSAIAAGHGDYALNVTGPILNFFAGHYDTPYPLPKSD
gi|6678664|      VSANGVQIGIWARPSAIDEGQGDYALNVTGPILNFFAQHYNTSYPLPKSD
gi|1351929|      QSPNNVQIRIWARPSAISEGHGQYALNVTGPILNFFANHYNTPYPLEKSD
gi|16877511|     VSANGVQIGIWARPSAIDEGQGDYALNVTGPILNFFAQHYNTSYPLPKSD
gi|4502095|      QASNGVLIRIWARPSAIAAGHGDYALNVTGPILNFFAGHYDTPYPLPKSD
gi|113743|       QASNGVLIRIWARPSAIAAGHGDYALNVTGPILNFFAGHYDTPYPLPKSD

                    360       370       380       390       400
                 ....|....|....|....|....|....|....|....|....|....|
SEC7             QIGLPDFNAGAMENWGLVTYRENSLLFDPLSSSSSNKERVVTVIAHELAH
gi|6678664|      QIALPDFNAGAMENWGLVTYRESSLMFDSQSSSISNKERVVTVIAHELAH
gi|1351929|      QIGLPDFNAGAMENWGLVTYRESALLFDPLVSSISNKERVVTVVAHELAH
gi|16877511|     QIALPDFNAGAMENWGLVTYRESSLMFDSQSSSISNKERVVTVIAHELAH
gi|4502095|      QIGLPDFNAGAMENWGLVTYRENSLLFDPLSSSSSNKERVVTVIAHELAH
gi|113743|       QIGLPDFNAGAMENWGLVTYRENSLLFDPLSSSSSNKERVVTVIAHELAH

                    410       420       430       440       450
                 ....|....|....|....|....|....|....|....|....|....|
SEC7             QWFGNLVTIEWWNDLWLNEGFASYVEYLGADYAEPTWNLKDLMVLNDVYR
gi|6678664|      QWFGNLVTVAWWNDLWLNEGFASYVEYLGADYAEPTWNLKDLMVLNDVYR
gi|1351929|      QWFGNLVTVDWWNDLWLNEGFASYVEYLGADYAEPTWNLKDLIVLNELHS
gi|16877511|     QWFGNLVTVAWWNDLWLNEGFASYVEYLGADYAEPTWNLKDLMVLNDVYR
gi|4502095|      QWFGNLVTIEWWNDLWLNEGFASYVEYLGADYAEPTWNLKDLMVLNDVYR
gi|113743|       QWFGNLVTIEWWNDLWLNEGFASYVEYLGADYAEPTWNLKDLMVLNDVYR

                    460       470       480       490       500
                 ....|....|....|....|....|....|....|....|....|....|
SEC7             VMAVDALASSHPLSTPASEINTPAQISELFDAISYSKGASVLRMLSSFLS
gi|6678664|      VMAVDALASSHPLSSPADEIKTPDQIMELFDSITYSKGASVLRMLSSFLT
gi|1351929|      VMAVDALASSHPLSSPADEMNTPAQISELFDSITYSKGASVLRMLSSFLT
gi|16877511|     VMAVDALASSHPLSSPADEIKTPDQIMELFDSITYSKGASVLRMLSSFLT
gi|4502095|      VMAVDALASSHPLSTPASEINTPAQISELFDAISYSKGASVLRMLSSFLS
gi|113743|       VMAVDALASSHPLSTPASEINTPAQISELFDAISYSKGASVLRMLSSFLS

                    510       520       530       540       550
                 ....|....|....|....|....|....|....|....|....|....|
SEC7             EDVFKQGLASYLHTFAYQNTIYLNLWDHLQEAVNNR-SIQLPTTERDIMN
gi|6678664|      EDLFKKGLSSYLHTYQYSNTMYLDLWEHLQKAVNQQTAVQPPATVRTIMD
gi|1351929|      EDLFKEGLASYLHTFAYQNTIYLDLWEHLQQAVNSQSAIQLPASVRDIMD
gi|16877511|     EDLFKKGLSSYLHTYQYSNTMYLDLWEHLQKAVNQQTAVQLPATVRTIMD
gi|4502095|      EDVFKQGLASYLHTFAYQNTIYLNLWDHLQEAVNNR-SIQLPTTVRDIMN
gi|113743|       EDVFKQGLASYLHTFAYQNTIYLNLWDHLQEAVNNR-SIQLPTTERDIMN

                    560       570       580       590       600
                 ....|....|....|....|....|....|....|....|....|....|
SEC7             RWTLQMGFPVITVDTSTGTLSQEHFLLDPDSNVTRPSEFNYWIVPITSI
gi|6678664|      RWILQMGFPVITVNTNTGEISQKHFLLDSKSNVTRPSEFNYWIAPIPFI
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gi|1351929|    RWILQMGFPVMTVNTINGIISQHHFLLDPTSNVTRPSDFNYLWIVPVSSM
gi|16877511|   RWILQMGFPVITVNTSTGEISQKHFLLDSKSNVTRPSEFNYIWIAPIPFL
gi|4502095|    RWTLQMGFPVITVDTSTGTLSQEHFLLDPDSNVTRPSEFNYMWIVPITSI
gi|113743|     RWTLQMGFPVITVDTSTGTLSQEHFLLDPDSNVTRPSEFNYMWIVPITSI
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                     610       620       630       640       650
               ...|....|....|....|....|....|....|....|....|....|
SEC7           RDGRQQQDYWLMDVR-AQNDLFSTSG-NEWVLLNLNVTGYYRVNYDENW
gi|6678664|    KSG-QEDHYWLDVEK-NQSAKFQTSS-NEWLLLNINVTGYYLVNYDENNW
gi|1351929|    RNGVLEQEEWLEGVEQTQNSLFRVEGDNNWILANLNVTGYYQVNYDEGNW
gi|16877511|   KSG-QEDHYWLDVEK-NQSAKFQTSS-NEWILLNINVTGYYLVNYDENNW
gi|4502095|    RDGRQQQDYWLIDVR-AQNDLFSTSG-NEWVLNLNVTGYYRVNYDEENW
gi|113743|     RDGRQQQDYWLMDVR-AQNDLFSTSG-NEWVLLNLNVTGYYRVNYDEENW
```

```
                     660       670       680       690       700
               ...|....|....|....|....|....|....|....|....|....|
SEC7           RKIQTQLQRDHSAIPVINRAQIINDAFNLASAHKVPVTLALNNTLFLIEE
gi|6678664|    KKLQNQLQTDLSVIPVINRAQIIEDSFNLASAKMIPTLALDNTLFLVKE
gi|1351929|    KKLQTQLQTNPSVIPVINRAQIIEDAFNLASAQKVPVTLALDNTLFLIRE
gi|16877511|   KKLQNQLQTDLSVIPVINRAQIIEDSFNLASAKMIPTLALDNTLFLVKE
gi|4502095|    RKIQTQLQRDHSAIPVINRAQIINDAFNLASAHKVPVTLALNNTLFLIEE
gi|113743|     RKIQTQLQRDHSAIPVINRAQIINDAFNLASAHKVPVTLALNNTLFLIEE
```

```
                     710       720       730       740       750
               ...|....|....|....|....|....|....|....|....|....|
SEC7           RCYMPWEAALSSLSYFKLMFDRSEVYGPMKNYLKKQVTPLFIHFRNNTNN
gi|6678664|    AEYMPWQAALSSLNYFTLMFDRSEVYGPMKRYLKKQVTPLFFYFQNRTNN
gi|1351929|    TEYMPWQAALSSLNYFKLMFDRSEVYGPMKNYLSKQVRPLFEHFKNITND
gi|16877511|   TEYMPWQAALSSLNYFTLMFDRSEVYGPMKRYLKKQVMPLFFYFQNRTNN
gi|4502095|    RCYMPWEAALSSLSYFKLMFDRSEVYGPMKNYLKKQVTPLFIHFRNNTNN
gi|113743|     RCYMPWEAALSSLSYFKLMFDRSEVYGPMKNYLKKQVTPLFIHFRNNTNN
```

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                     760       770       780       790       800
               ...|....|....|....|....|....|....|....|....|....|
SEC7           WREIPENLMDQYSEVNAISTACSNGVPECEEMVSGLFKQWMENPNNNPIH
gi|6678664|    WVNRPPTLMEQYNELNAISTACSSGLKECRDLVVELXSQWMKNPNNNTIH
gi|1351929|    WTRRPDTLMDQYNELNAISTACSNGIQECETLVSDLFKQWMDDPSNNPIH
gi|16877511|   WVNRPPTLMEQYNELNAISTACSSGLKECRDLVVELXSQWMKNPNNNTIH
gi|4502095|    WREIPENLMDQYSEVNAISTACSNGVPECEEMVSGLFKQWMENPNNNPIH
gi|113743|     WREIPENLMDQYSEVNAISTACSNGVPECEEMVSGLFKQWMENPNNNPIH
```

```
                     810       820       830       840       850
               ...|....|....|....|....|....|....|....|....|....|
SEC7           PNLRSTVYCNAIAQGGEEEWDFAWEQFRNATLVNEADKLRAALACSKELW
gi|6678664|    PNLRSTVYCNAIAFGGEEEWNFAWEQFRNATLVNEADKLRSALACSKDVW
gi|1351929|    PNLRTTVYCNAIALGGEREWDFAWEQFRNATLVNEADKLRSALACSNEMW
gi|16877511|   PNLRSTVYCNAIAFGGEEEWNFAWEQFRNATLVNEADKLRSALACSKDVW
gi|4502095|    PNLRSTVYCNAIAQGGEEEWDFAWEQFRNATLVNEADKLRAALACSKELW
gi|113743|     PNLRSTVYCNAIAQGGEEEWDFAWEQFRNATLVNEADKLRAALACSKELW
```

```
                     860       870       880       890       900
               ...|....|....|....|....|....|....|....|....|....|
SEC7           ILNRYLSYTLNPDLIRKQDATSTIISITNNVIGQGLVWDFVQSNWKKPFN
gi|6678664|    ILNRYLSYTLNPDYIRKQDTTSTIISIASNVAGHPLVWDFVRSNWKKLFE
gi|1351929|    ILNRYLSYTLNPDYIRQDATTSTINSIASNVAGQTLVWDFVQSNWKKLFE
gi|16877511|   ILNRYLSYTLNPDYIRKQDTTSTIISIASNVAGHPLVWDFVRSNWKKLFE
gi|4502095|    ILNRYLSYTLNPDLIRKQDATSTIISITNNVIGQGLVWDFVQSNWKKLFN
gi|113743|     ILNRYLSYTLNPDLIRKQDATSTIISITNNVIGQGLVWDFVQSNWKKPFN
```

```
                     910       920       930       940       950
               ...|....|....|....|....|....|....|....|....|....|
SEC7           DYGGGSFSFSNLIQAVTRRFSTEYELQQLEQFKKDNEETGFGSGTRALEQ
gi|6678664|    NYGGGSFSFANLIQGVTRRFSSEELQQLEQFKADNSATGFGIGTRALEQ
gi|1351929|    DEGGGSFSFANLIRAVTRRFSSEELQQLEQFRLNNLDTGFGSGTRALEQ
gi|16877511|   NYGGGSFSFANLIQGVTRRFSSEELQQLEQFKADNSATGFGIGTRALEQ
gi|4502095|    DYGGGSFSFSNLIQAVTRRFSTEYELQQLEQFKKDNEETGFGSGTRALEQ
gi|113743|     DYGGGSFSFSNLIQAVTRRFSTEYELQQLEQFKKDNEETGFGSGTRALEQ
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                     960       970
```

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         SEC7         ALEKTKANIKWVKENKEVVLQWFTENSK
         gi|6678664|  ALEKTRANIDWVKENKDAVFKWFTENSS
         gi|1351929|  ALEGTRANIKWVGENKEAVLAWFTANSA
         gi|16877511| ALEKTRANIDWVKENKDAVFKWFTENSG
         gi|4502095|  ALEKTKANIKWVKENKEVVLQWFTENSK
         gi|113743|   ALEKTKANIKWVKENKEVVLQWFTENSK
```

The presence of identifiable domains in SEC7, as well as all other SECX proteins, was determined by searches using software algorithms such as PROSITE, DOMAIN, Blocks, Pfam, ProDomain, and Prints, and then determining the Interpro number by crossing the 5 domain match (or numbers) using the Interpro website (http:www.ebi.ac.uk/ interpro). DOMAIN results for SEC7 as disclosed in Table 7F, were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST analyses. This BLAST analysis software samples domains found in the Smart and Pfam collections. Fully conserved single residues are indicated by the sign (|) and "strong" semi-conserved residues are indicated 10 by the sign (+). The "strong" group of conserved amino acid residues may be any one of the following groups of amino acids: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

Table 7F lists the domain description from DOMAIN analysis results against SEC7. This indicates that the SEC7 sequence has properties similar to those of other proteins known 15 to contain this domain.

Table 7F. Domain Analysis of SEC7
gnl|Pfam|pfam01433, Peptidase_M1, Peptidase family M1. Members of this family are aminopeptidases. The members differ widely in specificity, hydrolysing acidic, basic or neutral N-terminal residues. This family includes leukotriene-A4 hydrolase, this enzyme also has an aminopeptidase activity.
CD-Length = 393 residues, 100.0% aligned Score = 461 bits (1186), Expect = 9e-131

```
SEC 7:   76    LPNTLKPDSYQVTLRPYLTPNDRGLYVFKGSSTVRFTCK-EATDVIIIHSKKLNYTLSQG   134
               LP  +  P  Y + L   TP       F GS T+       TD I++H+K L
Sbjct:   1     LPTNVVPIHYDLRL----TPFLPEKPTFSGSVTITLQATIAGTDEIVLHAKDLTI-----   51

SEC 7:   135   HRVVLRGVGGSQPPDIDKTELVEPTEYLVVHLKGSLVKDSQYEMDSEFEGELADDLAGFY   194
               V L GV GS P  ++   L + T+ L + L   SL     QY ++ ++ G++++D + GFY
Sbjct:   52    SSVTLVGVNGSTPESVE-FSLQDETQKLTITLPQSLSAGQQYTLEIDYTGKISDSMLGFY   110

SEC 7:   195   RSEYMEG--NVRKVVATTQMQAADARKSFPCFDEPAMKAEFNITLIHPKDLTALSNMLPK   252
               RSEY +G     K +ATTQ +  DAR++FPCFDEP+ KA F IT+ HPK  TALSNM
Sbjct:   111   RSEYTDGGDGETKYMATTQFEPTDARRAFPCFDEPSFKATFTITITHPKGSTALSNMPVI   170
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SEC 7:   253  GPSTPLPEDPNWNVTEFHTTPKMSTYLLAFIVSEFDYVEKQASNGVLIRIWARPSAIAAG  312
              +     +D    +T F TTP MSTYLLAF+V +   Y+E +   +GV +R++ARP A  AG
Sbjct:   171  TTT---KDDDGRVITTFETTPPMSTYLLAFVVGDLTYLETETKDGVPVRVYARPGAKNAG  227

SEC 7:   313  HGDYALNVTGPILNFFAGHYDTPYPLPKSDQIGLPDFNAGAMENWGLVTYRENSLLFDPL  372
              G YAL+VT  +L F+  ++   PYPLPK DQ+ +PDF+AGAMENWGL+TYRE +LL+DP
Sbjct:   228  QGQYALDVTKKLLEFYEEYFGYPYPLPKLDQVAVPDFSAGAMENWGLITYREPALLYDPR  287

SEC 7:   373  SSSSSNKERVVTVIAHELAHQWFGNLVTIEWWNDLWLNEGFASYVEYLGAD--YAEPTWN  430
              SS++SNK+RV +VIAHELAHQWFGNLVT++WW+DLWLNEGFA+Y+EYL   D    EPTWN
Sbjct:   288  SSTNSNKQRVASVIAHELAHQWFGNLVTMKWWDDLWLNEGFATYLEYLITDELGGEPTWN  347

SEC 7:   431  LKDLMVLNDVYRVMAVDALASSHPLSTPASEINTPAQISELFDAISYSKG  480
              ++ L    +   +A DAL SSHP++    E+ TP++IS++FDAI+Y KG
Sbjct:   348  MEALF-GLVLQLALARDALGSSHPIT---VEVLTPSEISDIFDAITYEKG  393  (SEQ ID NO: 301)
```

The aminopeptidase-N (a/k/a APN, CD13, EC 3.4.11.2) disclosed herein as SEC7, is a well established marker of normal and malignant cells of the myelo-monocytic lineage. It is also expressed by leukaemic blasts of a small group of patients suffering from acute or chronic

5 lymphoid leukaemia. CD13/aminopeptidase N (E.C.3.4.11.2) is an ectoenzyme located in the outer membrane. A soluble, non-cell-associated form of CD13/GP150/aminopeptidase-N localizable to plasma also exists.

The expression of the APN gene in T cell lines as well as the induction of APN gene and surface expression in human peripheral T cells by mitogenic activation have been

10 demonstrated. For example, aminopeptidase expression was shown to be upregulated by a Th1-related cytokine, IFN-gamma. The induction of APN surface expression is partially resistent to the action of the inhibitors of protein biosynthesis, puromycin and cycloheximide, and is not prevented by tunicamycin, an inhibitor of glycosylation. The rapid mitogen-induced surface expression of APN, detectable 20 hours after stimulation, is dominated by mechanisms

15 not dependent on de novo protein biosynthesis or glycosylation. Monocyte, granulocyte, and lymphocyte-enriched cell fractions possess aminopeptidase-N activity that is inhibitable by CD13 antibodies. Immunoaffinity isolation of plasma aminopeptidase-N has also been carried out; further characterization using functional studies and sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) electrophoresis indicates that CD13 MABs can completely

20 clear plasma of aminopeptidase-N activity and that the purified protein has similar electrophoretic characteristics to cell-derived material.

The activity of aminopeptidase in, for example, bronchoalveolar lavage fluid (BALF) was significantly higher in patients with sarcoidosis than in normal volunteers (NV) and control patients (CP). The activity significantly correlated with lymphocyte percentages and

25 the ratio of CD4+ to CD8+ T lymphocytes in the BALF, and was higher in patients with sarcoidosis with parenchymal involvement than in those without the involvement. CD13/aminopeptidase N protein, which has a molecular mass of approximately 150 kD, was

detectable in alveolar macrophages (AM) from patients with sarcoidosis at higher levels than in those from NV. CD13/aminopeptidase N induced in vitro chemotactic migration of human lymphocytes in a concentration range of 10(-)(5) to 10(-)(1) U/ml. The chemotactic activity was greater for CD4+ T lymphocytes than for CD8+ T lymphocytes. The enzymatic activity of
5   CD13/aminopeptidase N was responsible for the chemotactic activity because bestatin, an inhibitor of CD13/aminopeptidase N, abolished the chemotactic activity. Higher chemotactic activity for lymphocytes was detected in the BALF from patients with sarcoidosis than in that from NV, and the activity was significantly decreased by treatment with bestatin. CD13/ aminopeptidase N expressed in AM thus has a role in T-lymphocyte involvement in the
10   sarcoid lung and the pathogenesis of alveolitis in this disorder and other disorders involving abberant cellular proliferation.

The SEC7 disclosed in this invention is expressed in at least the following tissues: apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus,
15   CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, those that express MHC I, II, and III, nervous tissues, medulla, subthalamic nucleus, ovary,
20   pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortia) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, public EST sources, literature sources, and/or RACE sources.
25   Further expression data for SEC7 is provided in Example 2.

The nucleic acids and proteins of SEC7 are useful in potential therapeutic applications implicated in various SEC-related pathological disorders described further herein. The SEC7 nucleic acid encoding the aminopeptidase-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic
30   acid or the protein are to be assessed.

These materials are further useful in the generation of antibodies that bind immunospecifically to the substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-SECX Antibodies" section

below. The disclosed SEC7 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, for example, a contemplated SEC7 epitope comprises from about amino acids 40 to about 120. In another embodiment, for example, a SEC7 epitope comprises from about amino acids 125 to about 275. In further embodiments,

5   for example, a SEC7 epitope comprises from about 280 to about 310, from about 320 to about 420, from about 430 to about 460, from about 470 to about 480, from about 500 to about 650, from about 660 to about 850, and from about 860 to about 961.

SEC8

10   The disclosed SEC8 (alternatively referred to herein as CG56010-01) includes the 398 nucleotide sequence (SEQ ID NO:15) shown in Table 8A. A SEC8 ORF begins with a Kozak consensus ATG initiation codon at nucleotides 2-4 and ends with a TAG codon at nucleotides 224-226.

Table 8A. SEC8 Nucleotide Sequence (SEQ ID NO:15)
GATGCTGGGGCTGGTCCTGGCCTTGCTGTCCTCCAGCTCTGCTGAGGAGTACGTGGGCCT GTCTGCAAACCAGTGTGCCGTGCCGGCCAAGGACAGGGTGGACTGCGGCTACCCCCATGT CACCCCCAAGGAGTGCAACAACCGGGGCTGCTGCTTTGACTCCAGGATCCCTGGAGTGCC TTGGTGTTTCAAGCCCCTGACTAGGAAGACAGAATGCACCTTCTGAGGCACCTCCAGCTG CCCCTGGGATGCAGGCTGAGCACCCTTGCCCGGCTGTGATTGCTGCCAGGCACTGTTCAT CTCAGTTTTTCTGTCCCTTTGCTCCCGGCAAGCTTTCTGCTGAAAGTTCATATCTGGAGC CTGATGTCTTAACGAATAAAGGTCCCATGCTCCACCCG

15   The SEC8 polypeptide (SEQ ID NO:16) encoded by SEQ ID NO:15 is 74 amino acids in length and is presented using the one-letter amino acid code in Table 8B. The Psort profile for SEC8 predicts that this sequence has a signal peptide and is likely to be secreted from the cell with a certainty of 0.3700. In alternative embodiments, a SEC8 polypeptide is located to

20   the endoplasmic reticulum (lumen) with a certainty of 0.1000, to the endoplasmic reticulum (membrane) with a certainty of 0.1000, or to lysosomes with a certainty of 0.1000. The Signal P predicts a likely cleavage site for a SEC8 peptide is between positions 14 and 15, i.e., at the dash in the sequence SSA-EE.

Table 8B. SEC8 protein sequence (SEQ ID NO:16)
MLGLVLALLSSSSAEEYVGLSANQCAVPAKDRVDCGYPHVTPKECNNRGCCFDSRIPGVP WCFKPLTRKTECTF

25

A BLAST analysis of SEC8 was run against the proprietary PatP GENESEQ Protein
Patent database. It was found, for example, that the amino acid sequence of SEC8 had high
homology to other proteins as shown in Table 8C.

Table 8C. BLASTX results from PatP database for SEC8		
Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum Probability P(N)
patp:AAR26876 Human intestinal trefoil factor	412	2.7e-38
patp:AAW27631 Human intestinal trefoil factor (hITF)	412	2.7e-38
patp:AAY99888 Human intestinal trefoil factor	412	2.7e-38
patp:AAW06550 Human colon specific gene CSG8	399	6.5e-37
patp:AAW46882 Protein sequence	399	6.5e-37

5

In a search of public sequence databases, it was found, for example, that the amino
acid sequence of the SEC8 protein of the present invention was found to have 74 of 74 amino
acid residues (100%) identical to the 74 amino acid NM_003226. SEC8 also has homology to
the proteins shown in the BLASTP data in Table 8D.

10

Table 8D. SEC8 BLASTP results					
Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positive (%)	Expect
gi|4507453|ref|NP_003217.1| (NM_003226)	trefoil factor 3 (intestinal); trefoil factor 3, HITF, human intestinal trefoil factor [Homo sapiens]	74	74/74 (100)	74/74 (100)	3e-27
gi|385570|gb|AAB27021.1|	trefoil factor [human, intestine, Peptide Partial, 61 aa]	61	61/61 (100)	61/61 (100)	1e-26
gi|7768743|dbj|BAA95531.1| (AP001746)	trefoil factor 3, HITF, human intestinal trefoil	74	70/74 (94)	72/74 (96)	3e-24
gi|17461336|ref|XP_032969.2| (XM_032969)	trefoil factor 3 (intestinal) [Homo sapiens]	80	70/74 (94)	72/74 (96)	5e-24
gi|12084578|pdb|1E9T|A	Chain A, High Resolution Solution Structure Of Human Intestinal	59	55/59 (93)	57/59 (96)	1e-22

This BLASTP data is displayed graphically in the ClustalW in Table 8E. A multiple
sequence alignment is given in Table 8E, with the SEC8 protein being shown on line 1, in a
ClustalW analysis comparing the protein of the invention with the related protein sequences
shown in Table 8D.

15

Table 8E. ClustalW Alignment of SEC8

```
1)SEC8 (SEQ ID NO:16)
2)gi|4507453| (SEQ ID NO:76)
3)gi|385570|  (SEQ ID NO:77)
4)gi|7768743| (SEQ ID NO:78)
5)gi|17461336| (SEQ ID NO:79)
6)gi|12084578| (SEQ ID NO:80)
```

```
                        10        20        30        40        50
                ....|....|....|....|....|....|....|....|....|....|
SEC8            -------MLGLVLALLSSSSAEEYVGLSANQCAVPAKDRVDCGYPHVTPK
gi|4507453|     -------MLGLVLALLSSSSAEEYVGLSANQCAVPAKDRVDCGYPHVTPK
gi|385570|      ---------------AEEYVGLSANQCAVPAKDRVDCGYPHVTPK
gi|7768743|     -------MLGLVLALLSSSSAEEYVGLSANQCAVPAKDRVDCGYPHVTPK
gi|17461336|    MAARALCMLGLVLALLSSSSAEEYVGLSANQCAVPAKDRVDCGYPHVTPK
gi|12084578|    -------------------XEYVGLSANQCAVPAKDRVDCGYPHVTPK
```

```
                        60        70        80
                ....|....|....|....|....|....|.
SEC8            ECNNRGCCFDSRIPGVPWCFKPLTRKTECTF
gi|4507453|     ECNNRGCCFDSRIPGVPWCFKPLTRKTECTF
gi|385570|      ECNNRGCCFDSRIPGVPWCFKPLTRKTECTF
gi|7768743|     ECNNRGCCFDSRIPGVPWCFKPLQ-EAECTF
gi|17461336|    ECNNRGCCFDSRIPGVPWCFKPLQ-EAECTF
gi|12084578|    ECNNRGCCFDSRIPGVPWCFKPLQ-EAECTF
```

The presence of identifiable domains in SEC8, as well as all other SECX proteins, was determined by searches using software algorithms such as PROSITE, DOMAIN, Blocks, Pfam, ProDomain, and Prints, and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (http:www.ebi.ac.uk/ interpro). DOMAIN results for SEC8 as disclosed in Table 8F, were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST analyses. This BLAST analysis software samples domains found in the Smart and Pfam collections. Fully conserved single residues are indicated by the sign (|) and "strong" semi-conserved residues are indicated by the sign (+). The "strong" group of conserved amino acid residues may be any one of the following groups of amino acids: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

Table 8F lists the domain description from DOMAIN analysis results against SEC8. This indicates that the SEC8 sequence has properties similar to those of other proteins known to contain this domain.

Table 8F. Domain Analysis of SEC8

gnl|Smart|smart00018, P, P or trefoil or TFF domain; Proposed role in renewal and pathology of mucous epithelia.
CD-Length = 47 residues, 95.7% aligned
Score = 62.4 bits (150), Expect = 9e-12

```
SEC 8:    23   NQCAVPAKDRVDCGYPHVTPKECNNRGCCFDSRIPGVPWCFKPLT   67
               QC+VP  +R++CG P +T  EC   RGCCFDS I GVPWCF P T
Sbjct:     1   FQCSVPPSERINCGPPGITEAECEARGCCFDSSISGVPWCFYPNT   45   (SEQ ID NO: 302)
```

TFF-peptides (i.e. TFF1, TFF2, TFF3; formerly P-domain peptides, trefoil factors) have been established as secretory products typical of mucin-producing epithelial cells, for example, the respiratory tract, the salivary glands, the uterus, and the conjunctiva. TFF-

5   peptides have a pivotal role in maintaining the surface integrity of these delicate epithelia as constituents of mucus gels as well as by their anti-apoptotic properties and their motogenic activity modulating cell migratory processes. Mucin-associated TFF-peptides (formerly P-domain peptides or trefoil factors) are typical motogens enhancing migration of cells in various in vitro models mimicking restitution of the intestine.

10   One of these peptides, TFF3, disclosed herein as SEC8 has been detected as a new neuropeptide of the human hypothalamo-pituitary axis where it is synthesized in oxytocinergic neurons of the paraventricular and supraoptic nuclei. From there it is transported to the posterior pituitary where it is released into the blood stream. Promoter methylation analyses showed that, in tissues where these genes are normally expressed, the proximal promoters of

15   TFF1 and TFF2 are specifically not methylated and that of TFF3 is partially demethylated. In contrast, in organs that do not express TFFs, the promoters of the three genes are methylated. These findings strongly argue for the involvement of epigenetic mechanisms in the regulation of TFF expression in normal and pathological conditions. In addition, TFF3 demonstrates anti-anoikic properties. TFF3 activates NF-kappaB in enterocytes, and TFF3-induced resistance to

20   anoikis in intestinal epithelial cells is mediated by a distinct signaling cascade linked to NF-kappaB.

Trefoil peptides are abundantly expressed epithelial cell products which exert protective effects and are key regulators of gastrointestinal epithelial restitution, the critical early phase of cell migration after mucosal injury. TFF-peptides act as motogens in the human

25   respiratory epithelium triggering rapid repair of damaged mucosa in the course of airway diseases such as asthma.   Synthesis of TFF-peptides also occurs pathologically as result to

chronic inflammatory diseases, for example of the gastrointestinal tract. Aberrant synthesis of TFF-peptides is observed in many tumors, for example, TTF is induced in human intestinal metaplasia and conserved in all gastric cancers.

The SEC8 disclosed in this invention is expressed in at least the following tissues:

5   apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver,

10   fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, those that express MHC I, II, and III, nervous tissues, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortia) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining

15   the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, public EST sources, literature sources, and/or RACE sources. Further expression data for SEC8 is provided in Example 2.

The nucleic acids and proteins of SEC8 are useful in potential therapeutic applications implicated in various SEC-related pathological disorders described further herein. The SEC8

20   nucleic acid encoding the TFF-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

These materials are further useful in the generation of antibodies that bind immunospecifically to the substances of the invention for use in therapeutic or diagnostic

25   methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-SECX Antibodies" section below. The disclosed SEC8 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, for example, a contemplated SEC8 epitope comprises from about amino acids 20 to about 65.

30

SEC9

The disclosed SEC9 (alternatively referred to herein as CG56162-01) includes the 1192 nucleotide sequence (SEQ ID NO:17) shown in Table 9A. A SEC9 ORF begins with a

Kozak consensus ATG initiation codon at nucleotides 151-153 and ends with a TGA codon at nucleotides 1091-1093.

Table 9A. SEC9 Nucleotide Sequence (SEQ ID NO:17)
CCAGCCCGAAAGGCAGGGTCTGGGTGCGGGAAGAGGGCTCGGAGCTGCCTTCCTGCTGCC TTGGGGCCGCCCAGATGAGGGAACAGCCCGATTTGCCTGGTTCTGATTCTCCAGGCTGTC GTGGTTGTGGAATGCAAACGCCAGCACATAATGGAAACAGGACCTGAAGACCCTTCCAGC ATGCCAGAGGAAAGTTCCCCCAGGCGGACCCCGCAGAGCATTCCCTACCAGGACCTCCCT CACCTGGTCAATGCAGACGGACAGTACCTCTTCTGCAGGTACTGGAAACCCACAGGCACA CCCAAGGCCCTCATCTTTGTGTCCCATGGAGCCGGAGAGCACAGTGGCCGCTATGAAGAG CTGGCTCGGATGCTGATGGGGCTGGACCTGCTGGTGTTCGCCCACGACCATGTTGGCCAC GGACAGAGCGAAGGGGAGAGGATGGTAGTGTCTGACTTCCACGTTTTCGTCAGGGATGTG TTGCAGCATGTGGATTCCATGCAGAAAGACTACCCTGGGCTTCCTGTCTTCCTTCTGGGC CACTCCATGGGAGGCGCCATCGCCATCCTCACGGCCGCAGAGAGGCCGGGCCACTTCGCC GGCATGGTACTCATTTCGCCTCTGGTTCTTGCCAATCCTGAATCTGCAACAACTTTCAAG GTCCTTGCTGCGAAAGTGCTCAACCTTGTGCTGCCAAACTTGTCCCTCGGGCCCATCGAC TCCAGCGTGCTCTCTCGGAATAAGACAGAGGTCGACATTTATAACTCAGACCCCCTGATC TGCCGGGCAGGGCTGAAGGTGTGCTTCGGCATCCAACTGCTGAATGCCGTCTCACGGGTG GAGCGCGCCCTCCCCAAGCTGACTGTGCCCTTCCTGCTGCTCCAGGGCTCTGCCGATCGC CTATGTGACAGCAAAGGGGCCTACCTGCTCATGGAGTTAGCCAAGAGCCAGGACAAGACT CTCAAGATTTATGAAGGTGCCTACCATGTTCTCCACAAGGAGCTTCCTGAAGTCACCAAC TCCGTCTTCCATGAAATAAACATGTGGGTCTCTCAAAGGACAGCCACGGCAGGAACTGCG TCCCCACCCTGAATGCATTGGCCGGTGCCCGGCTCATGGTCTGGGGGATGCAGGCAGGGG AAGGGCAGAGATGGCTTCTCAGATATGGCTTGCAAAAAAAAAAAAAAAAAAA

The SEC9 polypeptide (SEQ ID NO:18) encoded by SEQ ID NO:17 is 313 amino acids in length and is presented using the one-letter amino acid code in Table 9B. The Psort profile for SEC9 predicts that this sequence is likely to be localized at the cytoplasm with a certainty of 0.6500. In alternative embodiments, a SEC9 polypeptide is located to lysosomes (lumen) with a certainty of 0.1971, or to the mitochondrial matrix space with a certainty of 0.1000.

Table 9B. SEC9 protein sequence (SEQ ID NO:18)
METGPEDPSSMPEESSPRRTPQSIPYQDLPHLVNADGQYLFCRYWKPTGTPKALIFVSHG AGEHSGRYEELARMLMGLDLLVFAHDHVGHGQSEGERMVVSDFHVFVRDVLQHVDSMQKD YPGLPVFLLGHSMGGAIAILTAAERPGHFAGMVLISPLVLANPESATTFKVLAAKVLNLV LPNLSLGPIDSSVLSRNKTEVDIYNSDPLICRAGLKVCFGIQLLNAVSRVERALPKLTVP FLLLQGSADRLCDSKGAYLLMELAKSQDKTLKIYEGAYHVLHKELPEVTNSVFHEINMWV SQRTATAGTASPP

A BLAST analysis of SEC9 was run against the proprietary PatP GENESEQ Protein Patent database. It was found, for example, that the amino acid sequence of SEC9 had high homology to other proteins as shown in Table 9C.

Table 9C. BLASTX results from PatP database for SEC9

Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum Probability P(N)
patp:AAG10768 Arabidopsis thaliana protein fragment	343	5.6e-31
patp:AAG10769 Arabidopsis thaliana protein fragment	343	5.6e-31
patp:AAG10770 Arabidopsis thaliana protein fragment	343	5.6e-31
patp:AAB96388 Putative P. abyssi lysophospholipase	326	3.5e-29
patp:AAW23073 Thermococcus esterase CL-2-30LC	319	1.9e-28

In a search of public sequence databases, it was found, for example, that the amino acid sequence of the SEC9 protein of the present invention was found to have 313 of 313 amino acid residues (100%) identical to the 313 amino acid NM_007283. SEC9 also has

5   homology to the other proteins shown in the BLASTP data in Table 9D.

Table 9D. SEC9 BLASTP results					
Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positive (%)	Expect
gi|6005786|ref|NP_009214.1| (NM_007283)	monoglyceride lipase; lysophospholipase-like;	313	313/313/ (100)	313/313 (100)	e-179
gi|17440844|ref|XP_042586.2| (XM_042586)	lysophospholipase-like [Homo sapiens]	303	303/303 (100)	303/303 (100)	e-174
gi|6754690|ref|NP_035974.1| (NM_011844)	monoglyceride lipase [Mus musculus]	303	255/303 (84)	283/303 (93)	e-152
gi|2145125|gb|AAB58421.1| (U67964)	H14-E [Ectromelia virus]	277	132/272 (48)	184/272 (67)	1e-75
gi|17974944|ref|NP_536458.1| (NC_003310)	C5L [Monkeypox virus]	274	130/272 (47)	182/272 (66)	6e-74

This BLASTP data is displayed graphically in the ClustalW in Table 9E. A multiple sequence alignment is given in Table 9E, with the SEC9 protein being shown on line 1, in a

10   ClustalW analysis comparing the protein of the invention with the related protein sequences shown in Table 9D.

Table 9E. ClustalW Alignment of SEC9
1) SEC9   (SEQ ID NO:18) 2) gi|6005786| (SEQ ID NO:81) 3) gi|17440844| (SEQ ID NO:82) 4) gi|6754690| (SEQ ID NO:83) 5) gi|2145125| (SEQ ID NO:84) 6) gi|17974944| (SEQ ID NO:85)   10   20   30   40   50

```
                    ....|....|....|....|....|....|....|....|....|....|
        SEC9        METGPEDPSSMPEESSPRRTPQSIPYQDLPHLVNADGQYLFCRYWKPIGT
        gi|6005786| METGPEDPSSMPEESSPRRTPQSIPYQDLPHLVNADGQYLFCRYWKPIGT
        gi|17440844|----------MPEESSPRRTPQSIPYQDLPHLVNADGQYLFCRYWKPIGT
        gi|6754690| ----------MPEASSPRRTPQNWPYQDLPHLVNADGQYLFCRYWKPSGT
        gi|2145125| --------------MSAN------------CMFNLDNDYIYCKYWKPITY
        gi|17974944|--------------MSAN------------CMFNLDNDYIYCKYWKPITY

                             60        70        80        90       100
                    ....|....|....|....|....|....|....|....|....|....|
        SEC9        PKALIFVSHGAGEHSGRYEELARMLMGLDLLVFAHDHVGHGQSEGERMVV
        gi|6005786| PKALIFVSHGAGEHSGRYEELARMLMGLDLLVFAHDHVGHGQSEGERMVV
        gi|17440844|PKALIFVSHGAGEHSGRYEELARMLMGLDLLVFAHDHVGHGQSEGERMVV
        gi|6754690| PKALIFVSHGAGEHCGRYDELAHMLKGLDMLVFAHDHVGHGQSEGERMVV
        gi|2145125| PKALVFISHGAGEHSGRYDELAENISSLGILVFSHDHIGHGRSNGEKMMI
        gi|17974944|PKALVFISHGAGEHSGRYDELAENISSLGILVFSHDHIGHGRSNGEKMMI

                            110       120       130       140       150
                    ....|....|....|....|....|....|....|....|....|....|
        SEC9        SDFHVFVRDVLQHVDSMQKDYPGLPVFLLGHSMGGAIAILTAAERPGHFA
        gi|6005786| SDFHVFVRDVLQHVDSMQKDYPGLPVFLLGHSMGGAIAILTAAERPGHFA
        gi|17440844|SDFHVFVRDVLQHVDSMQKDYPGLPVFLLGHSMGGAIAILTAAERPGHFA
        gi|6754690| SDFQVFVRDVLQHVDTIQKDYPDVPIFLLGHSMGGAISILVAAERPTYFS
        gi|2145125| DDFGTYVRDVVQHVVIKSTYPGVPVFLLGHSMGATISILAAYENPNLFT
        gi|17974944|DDFGKYVRDVVQHVVIKSTYPGVPVFLLGHSMGATISILAACDNPNLFT

                            160       170       180       190       200
                    ....|....|....|....|....|....|....|....|....|....|
        SEC9        GMVLISPLVLANPESATTFKVLAAKVLNLVLPNISLGPIDSSVLSRNKIE
        gi|6005786| GMVLISPLVLANPESATTFKVLAAKVLNLVLPNISLGPIDSSVLSRNKIE
        gi|17440844|GMVLISPLVLANPESATTFKVLAAKVLNLVLPNISLGPIDSSVLSRNKSE
        gi|6754690| GMVLISPLVLANPESASTLKVLAAKLLNFVLPNMLGRIDSSVLSRNKSE
        gi|2145125| AMILMSPLVNAD--AVPRLNILAAKIMGTITPNVSVGKICPESVSRDKDE
        gi|17974944|AMILMSPLVNAD--AVPRLNILAAKIMGTITPNASVGKICPESVSRDMDE

                            210       220       230       240       250
                    ....|....|....|....|....|....|....|....|....|....|
        SEC9        VDIYNSDPLICRAGLKVCFGIQLLNAVSRVERALPKLTVPFLLLQGSADR
        gi|6005786| VDIYNSDPLICRAGLKVCFGIQLLNAVSRVERALPKLTVPFLLLQGSADR
        gi|17440844|VDIYNSDPLICRAGLKVCFGIQLLNAVSRVERALPKLTVPFLLLQGSADR
        gi|6754690| VDLYNSDPLVCRAGLKVCFGIQLLNAVRVERAIPRLTIPFLLLQGSADR
        gi|2145125| VYKYQYDPLVNHEKTKAGFASQVLKATNKVRKIIPKINTPTLILQGTNNE
        gi|17974944|VYKYQYDPLVNHEKTKAGFASQVLKATNKVRKIIPKIDTPTLILQGTNNE

                            260       270       280       290       300
                    ....|....|....|....|....|....|....|....|....|....|
        SEC9        LCDSKGAYLLMELAKSQDKTLKIYEGAYHVLHKELPEVTNSVFHEINMWV
        gi|6005786| LCDSKGAYLLMELAKSQDKTLKIYEGAYHVLHKELPEVTNSVFHEINMWV
        gi|17440844|LCDSKGAYLLMELAKSQDKTLKIYEGAYHVLHKELPEVTNSVFHEINMWV
        gi|6754690| LCDSKGAYLLMESSRSQDKTLKMYEGAYHVLHRELPEVTNSVLHEVNSWV
        gi|2145125| ISDVSGAYYFMQHANCN-REIKIYEGAKHHLHKETDEVKKSVMKEIETWI
        gi|17974944|ISDVSGAYYFMQHANCN-REIKIYEGAKHHLHKETDEVKKSVMKEIETWI

                            310
                    ....|....|...
        SEC9        SQRTATAGTASPP
        gi|6005786| SQRTATAGTASPP
        gi|17440844|SQRTATAGTASPP
        gi|6754690| SHRIAAAGAGCPP
        gi|2145125| FNRVKL-------
        gi|17974944|FNRVK--------
```

The presence of identifiable domains in SEC9, as well as all other SECX proteins, was determined by searches using software algorithms such as PROSITE, DOMAIN, Blocks, Pfam, ProDomain, and Prints, and then determining the Interpro number by crossing the

domain match (or numbers) using the Interpro website (http:www.ebi.ac.uk/ interpro).
DOMAIN results for SEC9 as disclosed in Table 9F, were collected from the Conserved
Domain Database (CDD) with Reverse Position Specific BLAST analyses. This BLAST
analysis software samples domains found in the Smart and Pfam collections. Fully conserved

5   single residues are indicated by the sign (|) and "strong" semi-conserved residues are indicated
by the sign (+). The "strong" group of conserved amino acid residues may be any one of the
following groups of amino acids: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY,
FYW.

       Table 9F lists the domain description from DOMAIN analysis results against SEC9.

10   This indicates that the SEC9 sequence has properties similar to those of other proteins known
to contain this domain.

Table 9F. Domain Analysis of SEC9
gnl|Pfam|pfam00561, abhydrolase, alpha/beta hydrolase fold. This catalytic domain is found in a very wide range of enzymes. CD-Length = 226 residues, 99.1% aligned Score = 63.9 bits (154), Expect = 1e-11

```
SEC 9:   82   VFAHDHVGHGQSEGERMVVSDFHVFVRDVLQHVDSMQKDYPGLPVFLLGHSMGGAIAILT   141
              V    D  G GQS    +     F     D+   +D++   D      V L+GHSMGGAIA
Sbjct:    3   VILFDLRGFGQSSPSDLAEYRFDDLAEDLEALLDALGLD----KVILVGHSMGGAIAAAY    58

SEC 9:  142   AAERPGHFAGMVLISP---LVLANPE-----------SATTFKVLAAKVLNLVLPNLSLG   187
              AA+  P     +VL+S      +L++                +    + + + L
Sbjct:   59   AAKYPERVKALVLVSAPHPALLSSRLFPRNLFGLLLANFRNRLLRSVEALLGRALKQFFL   118

SEC 9:  188   PIDSSVLSRNKTEVDIYNSDPLICRAGLKVCFGIQLLNAVSRVERALPKLTVPFLLLQGS   247
                  V     K            G  + +         V    L ++ VP L++ G
Sbjct:  119   LGRPLVSDFLKQFELSSLIRFGEDDGGDGLLWVALGKLLQWDVSADLKRIKVPTLVIWGD   178

SEC 9:  248   ADRLCDSKGAYLLMELAKSQDKTLKIYEGAYHVLHKELPEVTNSVFHEINMWV   300
              D L     + L L +     + + + A H+    E PE    V  I  ++
Sbjct:  179   DDPLVPPDASEKLSALFPNA--EVVVIDDAGHLAQLEKPEE---VAELILKFL   226   (SEQ ID NO: 303)
```

      Lysophospholipase, disclosed herein as SEC9, is a critical enzyme that acta on

15   biological membranes to regulate the multifunctional lysophospholipids. Increased levels of
lysophospholipids are associated with a host of diseases. Low activity of key phospholipid
catabolic and anabolic enzymes in human substantia nigra results in reduced ability to repair
oxidative membrane damage, as may occur, for example, in Parkinson's disease and
Alzheimer's disease. Lysophospholipase is a major autocrystallizing constituent of human

20   eosinophils and basophils, comprising approximately 10% of the total cellular protein in these
granulocytes. Identification of the distinctive hexagonal bipyramidal crystals of CLC protein

in body fluids and secretions has long been considered a hallmark of eosinophil-associated allergic inflammation. The compositions for and methods of modulating lysophospholipase has utility in the prevention and treatment of allergic diseases and inflammation.

The SEC9 disclosed in this invention is expressed in at least the following tissues: apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, those that express MHC I, II, and III, nervous tissues, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine, smooth muscle (coronary artery in aortia) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, public EST sources, literature sources, and/or RACE sources. Further expression data for SEC9 is provided in Example 2.

The nucleic acids and proteins of SEC9 are useful in potential therapeutic applications implicated in various SEC-related pathological disorders described further herein. The SEC9 nucleic acid encoding the lysophospholipase-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

These materials are further useful in the generation of antibodies that bind immunospecifically to the substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-SECX Antibodies" section below. The disclosed SEC9 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, for example, a contemplated SEC9 epitope comprises from about amino acids 10 to about 50. In another embodiment, for example, a SEC9 epitope comprises from about amino acids 55 to about 75. In further embodiments, for example, a SEC9 epitope comprises from about 90 to about 105, from about 110 to about 130, from about 180 to about 210, and from about 250 to about 313.

SEC10

The disclosed SEC10 (alternatively referred to herein as CG56164-01) includes the 1104 nucleotide sequence (SEQ ID NO:19) shown in Table 10A. A SEC10 ORF begins with a Kozak consensus ATG initiation codon at nucleotides 47-49 and ends with a TAG codon at

5   nucleotides 983-985. The disclosed SEC10 maps to human chromosome 17.

Table 10A. SEC10 Nucleotide Sequence (SEQ ID NO:19)
CTCGGTGCGCGACCCCGGCTCAGAGGACTCTTTGCTGTCCCGCAAGATGCGGATGCTGCT GGCGCTCCTGGCCCTCTCCGCGGCGCGGCCATCGGCCAGTGCAGAGTCACACTGGTGCTA CGAGGTTCAAGCCGAGTCCTCCAACTACCCCTGCTTGGTGCCAGTCAAGTGGGGTGGAAA CTGCCAGAAGGACCGCCAGTCCCCCATCAACATCGTCACCACCAAGGCAAAGGTGGACAA AAAACTGGGACGCTTCTTCTTCTCTGGCTACGATAAGAAGCAAACGTGGACTGTCCAAAA TAACGGGCACTCAGTGATGATGTTGCTGGAGAACAAGGCCAGCATTTCTGGAGGAGGACT GCCTGCCCCATACCAGGCCAAACAGTTGCACCTGCACTGGTCCGACTTGCCATATAAGGG CTCGGAGCACAGCCTCGATGGGGAGCACTTTGCCATGGAGATGCACATAGTACATGAGAG AGAGAAGGGGACATCGAGGAATGTGAAAGAGGCCCAGGACCCTGAAGACGAAATTGCGGT GCTGGCCTTTCTGGTGGAGGCTGGAACCCAGGTGAACGAGGGCTTCCAGCCACTGGTGGA GGCACTGTCTAATATCCCCAAACCTGAGATGAGCACTACGATGGCAGAGAGCAGCCTGTT GGACCTGCTCCCCAAGGAGGAGAAACTGAGGCACTACTTCCGCTACCTGGGCTCACTCAC CACACCGACCTGCGATGAGAAGGTCGTCTGGACTGTGTTCCGGGAGCCCATTCAGCTTCA CAGAGAACAGATCCTGGCATTCTCTCAGAAGCTGTACTACGACAAGGAACAGACAGTGAG CATGAAGGACAATGTCAGGCCCCTGCAGCAGCTGGGGCAGCGCACGGTGATAAAGTCCGG GGCCCCGGGTCGGCCGCTGCCCTGGGCCCTGCCTGCCCTGCTGGGCCCCATGCTGGCCTG CCTGCTGGCCGGCTTCCTGCGATGATGGCTCACTTCTGCACGCAGCCTCTCTGTTGCCTC AGCTCTCCAAGTTCCAGGCTTCCGGTCCTTAGCCTTCCCAGGTGGGACTTTAGGCATGAT TAAAATATGGACATATTTTTGGAG

The SEC10 polypeptide (SEQ ID NO:20) encoded by SEQ ID NO:19 is 312 amino acids in length and is presented using the one-letter amino acid code in Table 10B. The Psort

10   profile for SEC10 predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.9190. In alternative embodiments, a SEC10 polypeptide is located to lysosomal membranes with a certainty of 0.2000, to the endoplasmic reticulum (membrane) with a certainty of 0.1000, or to the endoplasmic reticulum (lumen) with a certainty of 0.1000. The Signal P predicts a likely cleavage site for a SEC10 peptide is

15   between positions 19 and 20, *i.e.*, at the dash in the sequence ASA-ES.

Table 10B. SEC10 protein sequence (SEQ ID NO:20)
MRMLLALLALSAARPSASAESHWCYEVQAESSNYPCLVPVKWGGNCQKDRQSPINIVTTK AKVDKKLGRFFFSGYDKKQTWTVQNNGHSVMMLLENKASISGGGLPAPYQAKQLHLHWSD LPYKGSEHSLDGEHFAMEMHIVHEKEKGTSRNVKEAQDPEDEIAVLAFLVEAGTQVNEGF QPLVEALSNIPKPEMSTTMAESSLLDLLPKEEKLRHYFRYLGSLTTPTCDEKVVWTVFRE PIQLHREQILAFSQKLYYDKEQTVSMKDNVRPLQQLGQRTVIKSGAPGRPLPWALPALLG PMLACLLAGFLR

A BLAST analysis of SEC10 was run against the proprietary PatP GENESEQ Protein Patent database. It was found, for example, that the amino acid sequence of SEC10 had high homology to other proteins as shown in Table 10C.

Table 10C. BLASTX results from PatP database for SEC10		
Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum Probability P(N)
patp:AAB59591 Human carbonic anhydrase isoform #4	1420	4.2e-145
patp:AAB54035 Human pancreatic cancer antigen	704	3.1e-69
patp:AAR91950 Lung cancer specific antigen HCAVIII	376	1.8e-34
patp:AAY96200 Non-small cell lung carcinoma cell antigen	376	1.8e-34
patp:AAY99460 Human PRO1335 amino acid sequence	376	1.8e-34

In a search of public sequence databases, it was found, for example, that the amino acid sequence of the SEC10 protein of the present invention was found to have 312 of 312 amino acid residues (100%) identical to the 312 amino acid NM_000717. SEC10 also has homology to the other proteins shown in the BLASTP data in Table 10D.

Table 10D. SEC10 BLASTP results					
Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positive (%)	Expect
gi|4502519|ref|NP_000708.1| (NM_000717)	carbonic anhydrase IV precursor; carbonic dehydratase [Homo sapiens]	312	312/312 (100)	312/312 (100)	e-175
gi|409725|gb|AAA35625.1| (L10955)	carbonic anhydrase IV [Homo sapiens]	294	294/312 (94)	294/312 (94)	e-160
gi|2554743|pdb|1ZNC|A	Chain A, Human Carbonic Anhydrase IV	266	266/266 (100)	266/266 (100)	e-155
gi|2134864|pir||S66253	carbonate dehydratase (EC 4.2.1.1) IV - human (fragments)	262	262/265 (98)	262/265 (98)	e-151
gi|17478944|ref|XP_008313.6| (XM_008313)	carbonic anhydrase IV precursor [Homo sapiens]	335	232/234 (99)	232/234 (99)	e-128

This BLASTP data is displayed graphically in the ClustalW in Table 10E. A multiple sequence alignment is given in Table 10E, with the SEC10 protein being shown on line 1, in a ClustalW analysis comparing the protein of the invention with the related protein sequences shown in Table 10D.

Table 10E. ClustalW

```
1) SEC10      (SEQ ID NO:20)
2) gi|4502519|  (SEQ ID NO:86)
3) gi|409725|   (SEQ ID NO:87)
4) gi|2554743|  (SEQ ID NO:88)
5) gi|2134864|  (SEQ ID NO:89)
6) gi|17478944| (SEQ ID NO:90)
                         10        20        30        40        50
                ....|....|....|....|....|....|....|....|....|....|
SEC10           --MRMLLALLALSAARPSASAESHWCYEVQAESSNYPCLVPVKWGGNCQK
gi|4502519|     --MRMLLALLALSAARPSASAESHWCYEVQAESSNYPCLVPVKWGGNCQK
gi|409725|      --MRMLLALLALSAARPSASA----------------VPVKWGGNCQK
gi|2554743|     AESHWCYEVQAESSNYP-CL-------------------VPVKWGGNCQK
gi|2134864|     AESHWCYEVQAESSNYP-------------------CLVPVKWGGNCQK
gi|17478944|    --MRMLLALXALSPARPSASAESHWCYEVQAESSNYPCLVPVKWGGNCQK

                         60        70        80        90       100
                ....|....|....|....|....|....|....|....|....|....|
SEC10           DRQSPINIVTTKAKVDKKLGRFFFSGYDKKQTWTVQNNGHSVMMLLENKA
gi|4502519|     DRQSPINIVTTKAKVDKKLGRFFFSGYDKKQTWTVQNNGHSVMMLLENKA
gi|409725|      DRQSPINIVTTKAKVDKKLGRFFFSGYDKKQTWTVQNNGHSVMMLLENKA
gi|2554743|     DRQSPINIVTTKAKVDKKLGRFFFSGYDKKQTWTVQNNGHSVMMLLENKA
gi|2134864|     DRQSPINIVTTKAKVDKKLGRFFFSGYDKKQTWTVQNNGHSVMMLLENKA
gi|17478944|    DRQSPINIVTTKAKVDKKLGRFFFSGYDKKQTWTVQNNGHSVMMLLENKA

                        110       120       130       140       150
                ....|....|....|....|....|....|....|....|....|....|
SEC10           SISGGGLPAPYQAKQLHLHWSDLPYKGSEHSLDGEHFAMEMHIVHEKEKG
gi|4502519|     SISGGGLPAPYQAKQLHLHWSDLPYKGSEHSLDGEHFAMEMHIVHEKEKG
gi|409725|      SISGGGLPAPYQAKQLHLHWSDLPYKGSEHSLDGEHFAMEMHIVHEKEKG
gi|2554743|     SISGGGLPAPYQAKQLHLHWSDLPYKGSEHSLDGEHFAMEMHIVHEKEKG
gi|2134864|     SISGGGLPAPYQAKQLHLHWSDLPYKGSEHSLDGEHFAMEMHIVHEKEKG
gi|17478944|    SISGGGLPAPYQAKQLHLHWSDLPYKGSEHSLDGEHFAMEMHIVHEKEKG

                        160       170       180       190       200
                ....|....|....|....|....|....|....|....|....|....|
SEC10           TSRNVKEAQDPEDEIAVLAFLVEAGTQVNEGFQPLVEALSNIPKPEMSTT
gi|4502519|     TSRNVKEAQDPEDEIAVLAFLVEAGTQVNEGFQPLVEALSNIPKPEMSTT
gi|409725|      TSRNVKEAQDPEDEIAVLAFLVEAGTQVNEGFQPLVEALSNIPKPEMSTT
gi|2554743|     TSRNVKEAQDPEDEIAVLAFLVEAGTQVNEGFQPLVEALSNIPKPEMSTT
gi|2134864|     TSRNVKEAQDPEDEIAVLAFLVEAGTQVNEGFQPLVEALSNIPKPEMSTT
gi|17478944|    TSRNVKEAQDPEDEIAVLAFLVEAGTQVNEGFQPLVEALSNIPKPEMSTT

                        210       220       230       240       250
                ....|....|....|....|....|....|....|....|....|....|
SEC10           MAESSLLDLLPKEEKLRHYFRYLGSLTTPTCDEKVVWTVF--------RE
gi|4502519|     MAESSLLDLLPKEEKLRHYFRYLGSLTTPTCDEKVVWTVF--------RE
gi|409725|      MAESSLLDLLPKEEKLRHYFRYLGSLTTPTCDEKVVWTVF--------RE
gi|2554743|     MAESSLLDLLPKEEKLRHYFRYLGSLTTPTCDEKVVWTVF--------RE
gi|2134864|     ---SSLLDLLPKEEKLRHYFRYLGSLTTPTCDEKVVWTVF--------RE
gi|17478944|    MAESSLLDLLPKEEKLRHYFRYLGSLTTPTCDEKVVXDCVPGAHSASQRT

                        260       270       280       290       300
                ....|....|....|....|....|....|....|....|....|....|
SEC10           PIQLHREQILAFSQKLYYD------KEQTVSMKDNVR-PLQQLGQRTVIK
gi|4502519|     PIQLHREQILAFSQKLYYD------KEQTVSMKDNVR-PLQQLGQRTVIK
gi|409725|      PIQLHREQILAFSQKLYYD------KEQTVSMKDNVR-PLQQLGQRTVIK
gi|2554743|     PIQLHREQILAFSQKLYYD------KEQTVSMKDNVR-PLQQLGQRTVIK
gi|2134864|     PIQLHREQILAFSQKLYYD------KEQTVSMKDNVR-PLQQLGQRTVIK
gi|17478944|    DPGILSEAVLRQGTDSEHEGQCQAPAAAGAAHGDKVRGPGSAAALGPACP

                        310       320       330
                ....|....|....|....|....|....|....|....|..
SEC10           SGAPGRPLPWALPA-----LLGPMLACLLAGFLR---
gi|4502519|     SGAPGRPLPWALPA-----LLGPMLACLLAGFLR---
gi|409725|      SGAPGRPLPWALPA-----LLGPMLACLLAGFLR---
gi|2554743|     S---------------------------------
gi|2134864|     ----------------------------------
```

gi|17478944| ACPECWPACWPASCNDASLDHAPSDLPQDSKDQASGP

The presence of identifiable domains in SEC10, as well as all other SECX proteins, was determined by searches using software algorithms such as PROSITE, DOMAIN, Blocks, Pfam, ProDomain, and Prints, and then determining the Interpro number by crossing the
5   domain match (or numbers) using the Interpro website (http:www.ebi.ac.uk/ interpro). DOMAIN results for SEC10 as disclosed in Table 10F, were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST analyses. This BLAST analysis software samples domains found in the Smart and Pfam collections. Fully conserved single residues are indicated by the sign (|) and "strong" semi-conserved residues are indicated
10 by the sign (+). The "strong" group of conserved amino acid residues may be any one of the following groups of amino acids: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

Table 10F lists the domain description from DOMAIN analysis results against SEC10. This indicates that the SEC10 sequence has properties similar to those of other proteins known
15 to contain this domain.

Table 10F. Domain Analysis of SEC10
gnl|Pfam|pfam00194, carb_anhydrase, Eukaryotic-type carbonic anhydrase.
CD-Length = 255 residues, 100.0% aligned
Score = 329 bits (844), Expect = 1e-91

```
SEC10:   23  WCYEVQAESSNYPCLVPVKWGGNCQKDRQSPINIVTTKAKVDKKLGRFFFSGYDKKQTWT  82
             W Y V   ++P L P+  G      DRQSPINI T KA+ D  L    S Y
Sbjct:    1  WGYGVHNGPEHWPLLYPIAGG-----DRQSPINIQTKKARYDPSLKPLSVSYYAATAK-E  54

SEC10:   83  VQNNGHSVMMLLEN---KASISGGGLPAPYQAKQLHLHWSDLPYKGSEHSLDGEHFAMEM  139
             + NNGHSV +  ++    K+ +SGG LPAPY+ KQ H HW      GSEH++DG  +  E+
Sbjct:   55  ITNNGHSVQVEFDDSMDKSVLSGGPLPAPYRLKQFHFHWGSSNEHGSEHTVDGVKYPAEL  114

SEC10:  140  HIVHEKEKGTSRNVKEAQDPEDEIAVLAFLVEAGTQVNEGFQPLVEALSNIPKPEMSTTM  199
             H+VH       + KEAQ  D +AVL  V+ G   N G Q LV+AL NI     S T
Sbjct:  115  HLVHWNS-TKYGSYKEAQKKPDGLAVLGVFVKVG-AENPGLQKLVDALQNIKTKGKSATF  172

SEC10:  200  AESSLLDLLPKEEKLRHYFRYLGSLTTPTCDEKVVWTVFREPIQLHREQILAFSQKLYYD  259
                 DLLP    LR Y+ Y GSLTTP C E V W V +EPI +  EQ+  F   L+
Sbjct:  173  TNFDPSDLLP---ALRDYWTYPGSLTTPPCTESVTWIVLKEPITVSSEQLEKFRSLLFSV  229

SEC10:  260  K-EQTVSMKDNVRPLQQLGQRTVIKS   284
             + E+ V M DN RP Q L  R V  S
Sbjct:  230  EGEEEVPMVDNYRPTQPLKGRVVRAS   255  (SEQ ID NO: 304)
```

Carbonic anhydrases, disclosed as SEC10 herein, are proteins involved in the catalytic hydration of carbon dioxide to carbonic acid. There is increasing evidence that hypoxia-
20 regulated gene expression influences tumor aggressiveness, contributing to the poorer outcome

of patients with hypoxic tumors. The role of the transcriptional complex hypoxia-inducible factor-1 as an important mediator of hypoxia-regulated gene expression is one of the best documented pathways. Recently, it has emerged that certain tumor-associated carbonic anhydrases (CAs) can be added to the list of known hypoxia-inducible factor-responsive

5   genes. CA expression in tumors with low vascularization defined a prognosis similar to the one of patients with highly angiogenic tumors. Multivariate analysis revealed that CA expression is a significant prognostic factor independent of angiogenesis. The expression of CA is linked to the expression of a constellation of proteins involved in angiogenesis, apoptosis inhibition, and cell-cell adhesion disruption, which explains the strong association of

10   CA with poor outcome.

       The SEC10 disclosed in this invention is expressed in at least the following tissues: apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells,

15   endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, those that express MHC I, II, and III, nervous tissues, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine,

20   smooth muscle (coronary artery in aortia) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, public EST sources, literature sources, and/or RACE sources. Further expression data for SEC10 is provided in Example 2.

25   The nucleic acids and proteins of SEC10 are useful in potential therapeutic applications implicated in various SEC-related pathological disorders described further herein. The SEC10 nucleic acid encoding the carbonic anhydrase-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

30   These materials are further useful in the generation of antibodies that bind immunospecifically to the substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-SECX Antibodies" section below. The disclosed SEC10 protein has multiple hydrophilic regions, each of which can be

used as an immunogen. In one embodiment, for example, a contemplated SEC10 epitope comprises from about amino acids 20 to about 100. In another embodiment, for example, a SEC10 epitope comprises from about amino acids 102 to about 160. In further embodiments, for example, a SEC10 epitope comprises from about 175 to about 290.

5

SEC11

The disclosed SEC11 (alternatively referred to herein as CG50379-01) includes the 2814 nucleotide sequence (SEQ ID NO:21) shown in Table 11A. A SEC11 ORF begins with a Kozak consensus ATG initiation codon at nucleotides 21-23 and ends with a TAG codon at

10   nucleotides 1764-1766. The SEC11 gene was mapped to human chromosome 12q24.33.

Table 11A. SEC11 Nucleotide Sequence (SEQ ID NO:21)
NGNACACGTCCAACGCCAGCATGCAGCGCCCGGGCCCCCGCCTGTGGCTGGTCCTGCAGG
TGATGGGCTCGTGCGCCGCCATCAGCTCCATGGACATGGAGCGCCCGGGCGACGGCAAAT
GCCAGCCCATCGAGATCCCGATGTGCAAGGACATCGGCTACAACATGACTCGTATGCCCA
ACCTGATGGGCCACGAGAACCAGCGCGAGGCAGCCATCCAGTTGCACGAGTTCGCGCCGC
TGGTGGAGTACGGCTGCCACGGCCACCTCCGCTTCTTCCTGTGCTCGCTGTACGCGCCGA
TGTGCACCGAGCAGGTCTCTACCCCCATCCCCGCCTGCCGGGTCATGTGCGAGCAGGCCC
GGCTCAAGTGCTCCCCGATTATGGAGCAGTTCAACTTCAAGTGGCCCGACTCCCTGGACT
GCCGGAAACTCCCCAACAAGAACGACCCCAACTACCTGTGCATGGAGGCGCCCAACAACG
GCTCGGACGAGCCCACCCGGGGCTCGGGCCTGTTCCCGCCGCTGTTCCGGCCGCAGCGGC
CCCACAGCGCGCAGGAGCACCCGCTGAAGGACGGGGGCCCCGGGCGCGGCGGCTGCGACA
ACCCGGGCAAGTTCCACCACGTGGAGAAGAGCGCGTCGTGCGCGCCGCTCTGCACGCCCG
GCGTGGACGTGTACTGGAGCCGCGAGGACAAGCGCTTCGCAGTGGTCTGGCTGGCCATCT
GGGCGGTGCTGTGCTTCTTCTCCAGCGCCTTCACCGTGCTCACCTTCCTCATCGACCCGG
CCCGCTTCCGCTACCCCGAGCGCCCCATCATCTTCCTCTCCATGTGCTACTGCGTCTACT
CCGTGGGCTACCTCATCCGCCTCTTCGCCGGCGCCGAGAGCATCGCCTGCGACCGGGACA
GCGGCCAGCTCTATGTCATCCAGGAGGGACTGGAGAGCACCGGCTGCACGCTGGTCTTCC
TGGTCCTCTACTACTTCGGCATGGCCAGCTCGCTGTGGTGGGTGGTCCTCACGCTCACCT
GGTTCCTGGCCGCCGGCAAGAAGTGGGGCCACGAGGCCATCGAAGCCAACAGCAGCTACT
TCCACCTGGCAGCCTGGGCCATCCCCGGCGGTGAAGACCATCCTGATCCTGGTCATGCGCA
GGGTGGCGGGGGACGAGCTCACCGGGGTCTGCTACGTGGGCAGCATGGACGTCAACGCGC
TCACCGGCTTCGTGCTCATTCCCCTGGCCTGCTACCTGGTCATCGGCACGTCCTTCATCC
TCTCGGGCTTCGTGGCCCTGTTCCACATCCGGAGGGTGATGAAGACGGGCGGCGAGAACA
CGGACAAGCTGGAGAAGCTCATGGTGCGTATCGGGCTCTTCTCTGTGCTGTACACCGTGC
CGGCCACCTGTGTGATCGCCTGCTACTTTTACGAACGCCTCAACATGGATTACTGGAAGA
TCCTGGCGGCGCAGCACAAGTGCAAAATGAACAACCAGACTAAAACGCTGGACTGCCTGA
TGGCCGCCTCCATCCCCGCCGTGGAGATCTTCATGGTGAAGATCTTTATGCTGCTGGTGG
TGGGGATCACCAGCGGGATGTGGATTTGGACCTCCAAGACTCTGCAGTCCTGGCAGCAGG
TGTGCAGCCGTAGGTTAAAGAAGAAGAGCCGGAGAAAACCGGCCAGCGTGATCACCAGCG
GTGGGATTTACAAAAAAGCCCAGCATCCCCAGAAAACTCACCACGGGAAATATGAGATCC
CTGCCCAGTCGCCCACCTGCGTGTGAACAGGGCTGGAGGGAAGGGCACAGGGGCGCCCGG
AGCTAAGATGTGGTGCTTTTCTTGGTTGTGTTTTTCTTTCTTCTTCTTCTTTTTTTTTT
TTTATAAAAGCAAAAGAGAAATACATAAAAAAGTGTTTACCCTGAAATTCAGGATGCTGT
GATACACTGAAAGGAAAAATGTACTTAAAGGGTTTTGTTTTGTTTTGGTTTTCCAGCGAA
GGGAAGCTCCTCCAGTGAAGTAGCCTCTTGTGTAACTAATTTGTGGTAAAGTAGTTGATT
CAGCCCTCAGAAGAAAACTTTTGTTTAGAGCCCTCCGTAAATATACATCTGTGTATTTGA
GTTGGCTTTGCTACCCATTTACAAATAAGAGGACAGATAACTGCTTTGCAAATTCAAGAG
CCTCCCCTGGGTTAACAAATGAGCCATCCCCAGGGCCCACCCCCAGGAAGGCCACAGTGC
TGGGCGGCATCCCTGCAGAGGAAAGACAGGACCCGGGGCCCGCCTCACACCCCAGTGGAT
TTGGAGTTGCTTAAAATAGACTCTGGCCTTCACCAATAGTCTCTCTGCAAGACAGAAACC
TCCATCAAACCTCACATTTGTGAACTCAAACGATGTGCAATACATTTTTTTCTCTTTCCT
TGAAAATAAAAAGAGAAACAAGTATTTTGCTATATATAAAGACAACAAAAGAAATCTCCT
AACAAAAGAACTAAGAGGCCCAGCCCTCAGAAACCCTTCAGTGCTACATTTTGTGGCTTT

```
TTAATGGAAACCAAGCCAATGTTATAGACGTTTGGACTGATTTGTGGAAAGGAGGGGGGA
AGAGGGAGAAGGATCATTCAAAAGTTACCCAAAGGGCTTATTGACTCTTTCTATTGTTAA
ACAAATGATTTCCACAAACAGATCAGGAAGCACTAGGTTGGCAGAGACACTTTGTCTAGT
GTATTCTCTTCACAGTGCCAGGAAAGAGTGGTTTCTGCGTGTGTATATTTGTAATATATG
ATATTTTTCATGCTCCACTATTTTATTAAAAATAAAATATGTTCTTTAAAAAAA
```

The SEC11 polypeptide (SEQ ID NO:22) encoded by SEQ ID NO:21 is 581 amino acids in length and is presented using the one-letter amino acid code in Table 11B. The Psort profile for SEC11 predicts that this sequence has a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. In alternative embodiments, a SEC11 polypeptide is located to the Golgi body with a certainty of 0.4600, to the endoplasmic reticulum (membrane) with a certainty of 0.3700, or to the endoplasmic reticulum (lumen) with a certainty of 0.1000. The Signal P predicts a likely cleavage site for a SEC11 peptide is between positions 20 and 21, *i.e.*, at the dash in the sequence CAA-IS.

Table 11B. SEC11 protein sequence (SEQ ID NO:22)
MQRPGPRLWLVLQVMGSCAAISSMDMERPGDGKCQPIEIPMCKDIGYNMTRMPNLMGHEN QREAAIQLHEFAPLVEYGCHGHLRFFLCSLYAPMCTEQVSTPIPACRVMCEQARLKCSPI MEQFNFKWPDSLDCRKLPNKNDPNYLCMEAPNNGSDEPTRGSGLFPPLFRPQRPHSAQEH PLKDGGPGRGGCDNPGKFHHVEKSASCAPLCTPGVDVYWSREDKRFAVVWLAIWAVLCFF SSAFTVLTFLIDPARFRYPERPIIFLSMCYCVYSVGYLIRLFAGAESIACDRDSGQLYVI QEGLESTGCTLVFLVLYYFGMASSLWWVVLTLTWFLAAGKKWGHEAIEANSSYFHLAAWA IPAVKTILILVMRRVAGDELTGVCYVGSMDVNALTGFVLIPLACYLVIGTSFILSGFVAL FHIRRVMKTGGENTDKLEKLMVRIGLFSVLYTVPATCVIACYFYERLNMDYWKILAAQHK CKMNNQTKTLDCLMAASIPAVEIFMVKIFMLLVVGITSGMWIWTSKTLQSWQQVCSRRLK KKSRRKPASVITSGGIYKKAQHPQKTHHGKYEIPAQSPTCV

A BLAST analysis of SEC11 was run against the proprietary PatP GENESEQ Protein Patent database. It was found, for example, that the amino acid sequence of SEC11 had high homology to other proteins as shown in Table 11C.

Table 11C. BLASTX results from PatP database for SEC11		
Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum Probability P(N)
patp:AAB73308 Human frizzled family protein 584	3151	0.0
patp:AAB73307 Mouse frizzled family protein 584	2971	1.8e-309
patp:AAY90903 Human frizzled-4 protein sequence	952	3.5e-149
patp:AAW31270 Mouse frizzled-4 protein Mfz4	949	4.5e-149
patp:AAB12117 Hydrophobic domain protein	1279	3.6e-130

In a search of public sequence databases, it was found, for example, that the amino acid sequence of the SEC11 protein of the present invention was found to have 581 of 581

amino acid residues (100%) identical to the 581 amino acid NM_007197. SEC11 also has homology to the other proteins shown in the BLASTP data in Table 11D.

Table 11D. SEC11 BLASTP results					
Gene Index / Identifier	**Protein / Organism**	**Length (aa)**	**Identity (%)**	**Positive (%)**	**Expect**
gi|6005762|ref|NP_009128.1| (NM_007197)	frizzled homolog 10 (Drosophila); frizzled (Drosophila) homolog 10 [Homo sapiens]	581	581/581 (100)	581/581 (100)	0.0
gi|17433077|sp|Q9PWH2|FZ10 CHICK	Frizzled 10 precursor (Frizzled-10) (Fz-10) (cFz-10)	585	502/564 (89)	531/564 (94)	0.0
gi|17433043|sp|Q9DEB5|FZOA XENLA	Frizzled 10A precursor (Frizzled-10A) (Fz-10A) (Xfz10A)	586	489/577 (84)	528/577 (90)	0.0
gi|17433096|sp|Q9W742|FZOB XENLA	Frizzled 10B precursor (Frizzled-10B) (Fz-10B) (Xfz10B) (Frizzled 9) (Xfz9)	580	482/564 (85)	521/564 (91)	0.0
gi|16508271|emb|CAD10102.1| (AL591180)	SC:dZ243A08.3 (frizzled homologue B) [Danio rerio]	580	437/566 (77)	493/566 (86)	0.0

This BLASTP data is displayed graphically in the ClustalW in Table 11E. A multiple sequence alignment is given in Table 11E, with the SEC11 protein being shown on line 1, in a ClustalW analysis comparing the protein of the invention with the related protein sequences shown in Table 11D.

Table 11E. ClustalW Alignment of SEC11

```
1)SEC11       (SEQ ID NO:22)
2)gi|6005762|    (SEQ ID NO:91)
3)gi|17433077|   (SEQ ID NO:92)
4)gi|17433043|   (SEQ ID NO:93)
5)gi|17433096|   (SEQ ID NO:94)
6)gi|16508271|   (SEQ ID NO:95)

                       10        20        30        40        50
               ....|....|....|....|....|....|....|....|....|....|
SEC11          MQRPG-----PRLWLVLQVMGS-CAAISSMDMRPGDGKCQPIEIPMCKD
gi|6005762|    MQRPG-----PRLWLVLQVMGS-CAAISSMDMRPGDGKCQPIEIPMCKD
gi|17433077|   MGPAAGN--LVRAVLAICWLAEHCAGISSIDIERPGDGRCQPIEIPMCKD
gi|17433043|   MDVSGVTGLLRGTALLLVLAAALCSAISSINPDRSGDGRCQAIEIPMCKD
gi|17433096|   MEPRVVT------ALLLSLAAALCSGISSINPDRSGEGRCQAIEIPMCKD
gi|16508271|   MFAAG-----VGISLGLLCFAGFCSAISSIDPDRPGEGRCQEIAIPLCKD

                       60        70        80        90        100
               ....|....|....|....|....|....|....|....|....|....|
SEC11          IGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCHGHLRFFLCSLYAPM
gi|6005762|    IGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCHGHLRFFLCSLYAPM
gi|17433077|   IGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCHGHLKFFLCSLYAPM
gi|17433043|   IGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCHSHLKFFLCSLYAPM
gi|17433096|   IGYNMTRMPNLMGHENQREAAIQLHEFAPLVEYGCHSHLKFFLCSLYAPM
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gi|16508271|    IGYNLTVMPNLMGHEDQNEAAIKLHEFAPLLEEGCHSHLKFFLCSLYAPM

                      110       120       130       140       150
                ....|....|....|....|....|....|....|....|....|....|
SEC11           CTEQVSTPIPACRVMCEQARLKCSPIMEQFNFKWPDSLDCRKLPNKNDPN
gi|6005762|     CTEQVSTPIPACRVMCEQARLKCSPIMEQFNFKWPDSLDCRKLPNKNDPN
gi|17433077|    CTEQVSTPIPACRVMCEQARLKCSPIMEQFNFKWPDSLDCSKLPNKNDPN
gi|17433043|    CTEQVSTPIPACRVMCEQARLKCSPIMEQFNFKWPDSLDCSKLPNKNDPN
gi|17433096|    CTEQVSTPIPACRVMCEQARLKCSPIMEQFNFKWPDSLDCSKLPNKNDPN
gi|16508271|    CTEQVSTPIPACRVMCEQARQKCSPIMEQFNFHWPESLDCSRLPNKNDPN

                      160       170       180       190       200
                ....|....|....|....|....|....|....|....|....|....|
SEC11           YLCMEAPNNGSDEPTRGSGLFPPLFRPQRPHSAQEHPLKDGGPGRGGCEN
gi|6005762|     YLCMEAPNNGSDEPTRGSGLFPPLFRPQRPHSAQEHPLKDGGPGRGGCEN
gi|17433077|    YLCMEAPNNGSDEPPRGSSMLPPMFRPQRPSTGHDLQQHKDSLSRTSCEN
gi|17433043|    YLCMEAPNNGIDETPRGSSMLPPLFRPQRPSSGHELYPKD-PTSRSSCEN
gi|17433096|    YLCMEAPNNGIDEAPRSSSILPPLFRPQRPNSGHEMYPKD-PKGRSSCEN
gi|16508271|    YLCMEAPNNGIDEPPKGSHTQSPDSRPPRPGNSQELPIKE-RVGKTCSN

                      210       220       230       240       250
                ....|....|....|....|....|....|....|....|....|....|
SEC11           PGKFHHVEKSASCAPLCTPGVDVYWSREDKRFAVVWLAIWAVLCFFSSAF
gi|6005762|     PGKFHHVEKSASCAPLCTPGVDVYWSREDKRFAVVWLAIWAVLCFFSSAF
gi|17433077|    PGKFHHVEKSASCAPLCTPGVDVYWSKDDKQFAVIWIAIWSLLCFFSSAF
gi|17433043|    SGKFHHVEKSASCAPLCSSSVDVYWSKDDKKFAFIWIAIWSLLCFFSSAF
gi|17433096|    SGKFHHVEKSASCAPLCSSSVDVYWSKNDKKFAFIWIAIWSLLCFFSSAF
gi|16508271|    PGKFHMVQKSESCAPKCYSNVDVYWSQGDKRFSMVWIAIWSILCFISSAF

                      260       270       280       290       300
                ....|....|....|....|....|....|....|....|....|....|
SEC11           TVLTFLIDPARFRYPERPIIFLSMCYCVYSVGYLIRLFAGAESIACDRDS
gi|6005762|     TVLTFLIDPARFRYPERPIIFLSMCYCVYSVGYLIRLFAGAESIACDRDS
gi|17433077|    TVLTFLIDPQRFKYPERPIIFLSMCYCVYSVGYIIRLFSGAESIACDRDS
gi|17433043|    TVLTFLVDPLRFKYPERPIIFLSMCYCVYSVGYIIRLFAGADSIACDRDS
gi|17433096|    TVLTFLVDPLRFKYPERPIIFLSMCYCVYSVGYIIRLFAGADSIACDRDS
gi|16508271|    TVLTFLIDPQRFKYPERPIIFLSMSYCVYSVGELVRLFVGVENVACDRDT

                      310       320       330       340       350
                ....|....|....|....|....|....|....|....|....|....|
SEC11           GQLYVIQEGLESTGCTIVFLVLYYFGMASSLWWVMLTLTWFLAAGKKWGH
gi|6005762|     GQLYVIQEGLESTGCTIVFLVLYYFGMASSLWWVMLTLTWFLAAGKKWGH
gi|17433077|    GQLYVIQEGLESTGCTIVFLVLYYFGMASSLWWVILTLTWFLAAGKKWGH
gi|17433043|    GQLYVIQEGLESTGCTIVFLILYYFGMASSLWWVILTLTWFLAAGKKWGH
gi|17433096|    GQLYVIQEGLESTGCTIVFLILYYFGMASSLWWVILTLTWFLAAGKKWGH
gi|16508271|    GVQYIIQEGLESTGCTIVFLILYYFGMASSLWWVILTLTWFLAAGKKWGH

                      360       370       380       390       400
                ....|....|....|....|....|....|....|....|....|....|
SEC11           EAIEANSSYFHLAAWAIPAVKTIEILVMRRVAGDELTGVCYVGSMDVNAL
gi|6005762|     EAIEANSSYFHLAAWAIPAVKTIEILVMRRVAGDELTGVCYVGSMDVNAL
gi|17433077|    EAIEANSSYFHLAAWAIPAVKTIMILVMRRVAGDELTGLCYVGSMDVNAL
gi|17433043|    EAIEANSSYFHLAAWAIPAVKTIMILVMRRVAGDELTGVCYVGSMDVNAL
gi|17433096|    EAIEANSSYFHLAAWAIPAVKTIMILVMRRVAGDELTGVCYVGSMDVNAL
gi|16508271|    EAIEANSSYFHLAAWAIPAIKTIMILVMRKVAGDELTGVCYVGSMDVKAL

                      410       420       430       440       450
                ....|....|....|....|....|....|....|....|....|....|
SEC11           TGFVLIPLACYLVIGTSFILSGFVALFHIRRVMKTGGENTDKLEKLMVRI
gi|6005762|     TGFVLIPLACYLVIGTSFILSGFVALFHIRRVMKTGGENTDKLEKLMVRI
gi|17433077|    TGFVLIPLACYLIIGTSFILSGFVALFHIRRVMKTGGENTDKLEKLMVRI
gi|17433043|    TGFVLIPLACYLIIGTSFILSGFVALFHIRRVMKTGGENTDKLEKLMVRI
gi|17433096|    TGFVLIPLACYLIIGTSFILSGFVALFHIRRVMKTGGENTDKLEKLMVRI
gi|16508271|    TGFVLIPLSCYLIIGTSFLLSGFVALFHIRKVMKTEGENTDKLEKLMVRI

                      460       470       480       490       500
                ....|....|....|....|....|....|....|....|....|....|
SEC11           GLFSVLYTVPATCVIACYFYERLNMDYWKILAAQHKCKMNNQTKTLDCLM
gi|6005762|     GLFSVLYTVPATCVIACYFYERLNMDYWKILAAQHKCKMNNQTKTLDCLM
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gi|17433077|  GVFSVLYTVPATCVIACYFYERLNMDYWKIMASQQKCKMNNQTKNLDCMM
gi|17433043|  GVFSVLYTVPATCVIACYFYERLNMDEWKILATQDKCKMDSQTKTLDCTM
gi|17433096|  GVFSVLYTVPATCVIACYFYERLNMDEWKILATQDKCKMDSQTKTLDCTM
gi|16508271|  GVFSVLYTVPATCVIACYFYERLNMDYWKILAGEQKCADDGKSG-EECMM

                    510       520       530       540       550
              ....|....|....|....|....|....|....|....|....|....|
SEC11         AASIPAVEIFMVKIFMLLVVGITSGMWIWTSKTLQSWQQVCSRRLKKKSR
gi|6005762|   AASIPAVEIFMVKIFMLLVVGITSGMWIWTSKTLQSWQQVCSRRLKKKSR
gi|17433077|  NNSIPAVEIFMVKIFMLLVVGITSGMWIWTSKTLQSWQNVCSRRLKKRSR
gi|17433043|  TSSIPAVEIFMVKIFMLLVVGITSGMWIWTSKTMQSWQNVFSKRLKKRNR
gi|17433096|  TSSIPAVEIFMVKIFMLLVVGITSGMWIWTSKTMQSWQNVFSKSLKKRNR
gi|16508271|  KSSIPAVEIFMVKIFMLLVVGITSGMWIWTSKTLQSWQNVFSRKLKKKQR

                    560       570       580
              ....|....|....|....|....|....|....|..
SEC11         RKPASVITSGGIYKKAQHPQKTHHGKYEIPAQSPTCV
gi|6005762|   RKPASVITSGGIYKKAQHPQKTHHGKYEIPAQSPTCV
gi|17433077|  RKPASVITSSGIYKKPQHPQKTHLAKYESTLQPPTCV
gi|17433043|  SKPASVITSAGIYKKPQHPPKVHHGKYESALQSPTCV
gi|17433096|  NKPASVITSAGIYKKPQQPPKIHHGKYESALRSPTCV
gi|16508271|  RKAACVFTGSGPYLKPHPALKGHKTKYEPAGPPATCV
```

The presence of identifiable domains in SEC11, as well as all other SECX proteins, was determined by searches using software algorithms such as PROSITE, DOMAIN, Blocks, Pfam, ProDomain, and Prints, and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (http:www.ebi.ac.uk/ interpro). DOMAIN results for SEC11 as disclosed in Table 11F, were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST analyses. This BLAST analysis software samples domains found in the Smart and Pfam collections. Fully conserved single residues are indicated by the sign (|) and "strong" semi-conserved residues are indicated by the sign (+). The "strong" group of conserved amino acid residues may be any one of the following groups of amino acids: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

Table 11F lists the domain description from DOMAIN analysis results against SEC11. This indicates that the SEC11 sequence has properties similar to those of other proteins known to contain this domain.

Table 11F. Domain Analysis of SEC11

<u>gnl|Pfam|pfam01534</u>, **Frizzled, Frizzled/Smoothened family membrane region. This family contains the membrane spanning region of frizzled and smoothened receptors. This membrane region is predicted to contain seven transmembrane alpha helices. Proteins related to drosophila frizzled are receptors for the Wnt signaling molecules. The smoothened receptor mediates hedgehog signaling.**

CD-Length = 328 residues, 98.2% aligned

Score = 418 bits (1075), Expect = 4e-118

```
SEC11:   217   VYWSREDKRFAVVWLAIWAVLCFFSSAFTVLTFLIDPARFRYPERPIIFLSMCYCVYSVG   276
               +SR++ RFA   W+A W+ LCF S+ FTVLTFLID  RFRYPERPI +LS CY + SVG
Sbjct:     1   PLFSRDEHRFARSWIAWWSALCFVSTLFTVLTFLIDWKRFRYPERPIFYLSACYLIVSVG    60

SEC11:   277   YLIRLFAGAESIACDR-DSGQLYVIQEGLESTGCTLVFLVLYYFGMASSLWWVVLTLTWF   335
               YLIR F G E IAC + D G   V Q   E+   CT++FL++Y+FGMASS+WWV+LTLTWF
Sbjct:    61   YLIRFFLGREEIACRKADGGMRTVTQGSTENLSCTVLFLLVYFFGMASSVWWVILTLTWF   120

SEC11:   336   LAAGKKWGHEAIEANSSYFHLAAWAIPAVKTILILVMRRVAGDELTGVCYVGSMDVNALT   395
               LAAG KWGHEAIEA SSYFHL AW +PAV TI +L + +V GD +TG+C+VG++++++AL
Sbjct:   121   LAAGLKWGHEAIEAKSSYFHLVAWGLPAVLTITVLALNKVDGDPITGICFVGNLNLDALR   180

SEC11:   396   GFVLIPLACYLVIGTSFILSGFVALFHIRRVMKTGGENTDKLEKLMVRIGLFSVLYTVPA   455
               GFVL PL   YLVIGT F+L+GFV+LF IR V+KT G NT KLEKLMVRIG+FS+LYTVPA
Sbjct:   181   GFVLAPLCVYLVIGTLFLLAGFVSLFRIRSVIKTQGTNTSKLEKLMVRIGVFSLLYTVPA   240

SEC11:   456   TCVIACYFYERLNMDYWKILAAQHKCK-----MNNQTKTLDCLMAASIPAVEIFMVKIFM   510
               VIACYFYE+ N D W+     C       + K+ D      P + +FM+K FM
Sbjct:   241   LIVIACYFYEQANRDEWERSWLDCICCQYQIPCPYKDKSSDPEAR---PPLAVFMLKYFM   297

SEC11:   511   LLVVGITSGMWIWTSKTLQSWQQVC   535
               LVVGITSG+W+W+ KTL+SW++
Sbjct:   298   SLVVGITSGVWVWSKKTLESWRRFF   322   (SEQ ID NO: 305)
```

Frizzled (FZD) genes, disclosed herein as SEC11, encode receptors for WNTs, which play key roles in carcinogenesis and embryogenesis. Homologues of the N-terminal region of frizzled exist either as transmembrane or secreted molecules. The secreted frizzled related

5   protein 2 (sFRP2) is upregulated within 2 days of in vitro development.   In vivo sFRP2 expression was likewise found in mesenchymal condensates and subsequent epithelial structures. Detailed in situ hybridization analysis revealed sFRP2 expression during development of the eye, brain, neural tube, craniofacial mesenchyme, joints, testis, pancreas and below the epithelia of oesophagus, aorta and ureter where smooth muscles develop. In a

10   comparative analysis, transcripts of the related sFRP1 and sFRP4 genes were frequently found in the same tissues as sFRP2 with their expression domains overlapping in some instances, but mutually exclusive in others. While sFRP1 is specifically expressed in the embryonic metanephros, eye, brain, teeth, salivary gland and small intestine, there is only weak expression of sFRP4 except for the developing teeth, eye and salivary gland.   Nevertheless,

15   sFRP genes play quite distinct roles in the morphogenesis of several organ systems.

The SEC11 disclosed in this invention is expressed in at least the following tissues: apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells,

5   endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye, frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, those that express MHC I, II, and III, nervous tissues, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine,

10   smooth muscle (coronary artery in aortia) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, public EST sources, literature sources, and/or RACE sources. Further expression data for SEC11 is provided in Example 2.

15   The nucleic acids and proteins of SEC11 are useful in potential therapeutic applications implicated in various SEC-related pathological disorders described further herein. The SEC11 nucleic acid encoding the FZD10-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

20   These materials are further useful in the generation of antibodies that bind immunospecifically to the substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-SECX Antibodies" section below. The disclosed SEC11 protein has multiple hydrophilic regions, each of which can be

25   used as an immunogen. In one embodiment, for example, a contemplated SEC11 epitope comprises from about amino acids 20 to about 85. In another embodiment, for example, a SEC11 epitope comprises from about amino acids 110 to about 200. In further embodiments, for example, a SEC11 epitope comprises from about 240 to about 260, from about 261 to about 290, from about 330 to 350, from about 420 to 430, from about 460 to 490, and about

30   520 to 581.

SEC12

The disclosed SEC12 (alternatively referred to herein as CG56035-01) includes the 2840 nucleotide sequence (SEQ ID NO:23) shown in Table 12A. A SEC12 ORF begins with a Kozak consensus ATG initiation codon at nucleotides 258-260 and ends with a TGA codon at nucleotides 1296-1298.

Table 12A. SEC12 Nucleotide Sequence (SEQ ID NO:23)

```
CAGCGGCCGCTGAATTCTAGGGCGGGTTCGCGCCCCGAAGGCTGAGAGCTGGCGCTGCTC
GTGCCCTGTGTGCCAGACGGCGGAGCTCCGCGGCCGGACCCCGCGGCCCCGCTTTGCTGC
CGACTGGAGTTTGGGGGAAGAAACTCTCCTGCGCCCCAGAAGATTTCTTCCTCGGCGAAG
GGACAGCGAAAGATGAGGGTGGCAGGAAGAGAAGGCGCTTTCTGTCTGCCGGGGTCGCAG
CGCGAGAGGGCAGTGCCATGTTCCTCTCCATCCTAGTGGCGCTGTGCCTGTGGCTGCACC
TGGCGCTGGGCGTGCGCGGCGCGCCCTGCGAGGCGGTGCGCATCCCTATGTGCCGGCACA
TGCCCTGGAACATCACGCGGATGCCCAACCACCTGCACCACAGCACGCAGGAGAACGCCA
TCCTGGCCATCGAGCAGTACGAGGAGCTGGTGGACGTGAACTGCAGCGCCGTGCTGCGCT
TCTTCTTCTGTGCCATGTACGCGCCCATTTGCACCCTGGAGTTCCTGCACGACCCTATCA
AGCCGTGCAAGTCGGTGTGCCAACGCGCGCGCGACGACTGCGAGCCCCTCATGAAGATGT
ACAACCACAGCTGGCCCGAAAGCCTGGCCTGCGACGAGCTGCCTGTCTATGACCGTGGCG
TGTGCATTTCGCCTGAAGCCATCGTCACGGACCTCCCGGAGGATGTTAAGTGGATAGACA
TCACACCAGACATGATGGTACAGGAAAGGCCTCTTGATGTTGACTGTAAACGCCTAAGCC
CCGATCGGTGCAAGTGTAAAAAGGTGAAGCCAACTTTGGCAACGTATCTCAGCAAAAACT
ACAGCTATGTTATTCATGCCAAAATAAAAGCTGTGCAGAGGAGTGGCTGCAATGAGGTCA
CAACGGTGGTGGATGTAAAAGAGATCTTCAAGTCCTCATCACCCATCCCTCGAACTCAAG
TCCCGCTCATTACAAATTCTTCTTGCCAGTGTCCACACATCCTGCCCCATCAAGATGTTC
TCATCATGTGTTACGAGTGGCGTTCAAGGATGATGCTTCTTGAAAATTGCTTAGTTGAAA
AATGGAGAGATCAGCTTAGTAAAAGATCCATACAGTGGGAAGAGAGAGGCTGCAGGAACAGC
GGAGAACAGTTCAGGACAAGAAGAAAACAGCCGGGCGCACCAGTCGTAGTAATCCCCCCA
AACCAAAGGGAAAGCCTCCTGCTCCCAAACCAGCCAGTCCCAAGAAGAACATTAAAACTA
GGAGTGCCCAGAAGAGAACAAACCCGAAAAGAGTGTGAGCTAACTAGTTTCCAAAGCGGA
GACTTCCGACTTCCTTACAGGATGAGGCTGGGCATTGCCTGGGACAGCCTATGTAAGGCC
ATGTGCCCCTTGCCCTAACAACTCACTGCAGTGCTCTTCATAGACACATCTTGCAGCATT
TTTCTTAAGGCTATGCTTCAGTTTTTCTTTGTAAGCCATCACAAGCCATAGTGGTAGGTT
TGCCCTTTGGTACAGAAGGTGAGTTAAAGCTGGTGGAAAAGGCTTATTGCATTGCATTCA
GAGTAACCTGTGTGCATACTCTAGAAGAGTAGGGAAAATAATGCTTGTTACAATTCGACC
TAATATGTGCATTGTAAAATAAATGCCATATTTCAAACAAAACACGTAATTTTTTTACAG
TATGTTTTATTACCTTTTGATATCTGTTGTTGCAATGTTAGTGATGTTTTAAAATGTGAT
GAAAATATAATGTTTTTAAGAAGGAACAGTAGTGGAATGAATGTTAAAAGATCTTTATGT
GTTTATGGTCTGCAGAAGGATTTTTGTGATGAAAGGGGATTTTTTGAAAAATTAGAGAAG
TAGCATATGGAAAATTATAATGTGTTTTTTTACCAATGACTTCAGTTTCTGTTTTTAGCT
AGAAACTTAAAAACAAAAATAATAATAAAGAAAAATAAATAAAAAGGAGAGGCAGACAAT
GTCTGGATTCCTGTTTTTTGGTTACCTGATTTCCATGATCATGATGCTTCTTGTCAACAC
CCTCTTAAGCAGCACCAGAAACAGTGAGTTTGTCTGTACCATTAGGAGTTAGGTACTAAT
TAGTTGGCTAATGCTCAAGTATTTTATACCCACAAGAGAGGTATGTCACTCATCTTACTT
CCCAGGACATCCACCCTGAGAATAATTTGACAAGCTTAAAAATGGCCTTCATGTGAGTGC
CAAATTTTGTTTTTCTTCATTTAAATATTTTCTTTGCCTAAATACATGTGAGAGGAGTTA
AATATAAATGTACAGAGAGGAAAGTTGAGTTCCACCTCTGAAATGAGAATTACTTGACAG
TTGGGATACTTTAATCAGAAAAAAAGAACTTATTTGCAGCATTTTATCAACAAATTTCAT
AATTGTGGACAATTGGAGGCATTTATTTTAAAAAACAATTTTATTGGCCTTTTGCTAACA
CAGTAAGCATGTATTTTATAAGGCATTCAATAAATGCACAACGCCCAAAGGAAATAAAAT
CCTATCTAATCCTACTCTCCACTACACAGAGGTAATCACTATTAGTATTTTGGCATATTA
TTCTCCAGGTGTTTGCTTATGCACTTATAAAATGATTTGAACAAATAAAACTAGGAACCT
GTATACATGTGTTTCATAACCTGCCTCCTTTGCTTGGCCCTTTATTGAGATAAGTTTTCC
TGTCAAGAAAGCAGAAACCATCTCATTTCTAACAGCTGTGTTATATTCCATAGTATGCAT
TACTCAACAAACTGTTGTGCTATTGGATACTTAGGTGGTTTCTTCACTGACAATACTGAA
TAAACATCTCACCGGAATTC
```

The SEC12 polypeptide (SEQ ID NO:24) encoded by SEQ ID NO:23 is 346 amino acids in length and is presented using the one-letter amino acid code in Table 12B. The Psort profile for SEC12 predicts that this sequence has a signal peptide and is likely to be secreted from the cell with a certainty of 0.8200. In alternative embodiments, a SEC12 polypeptide is located to lysosomes with a certainty of 0.1900, or to the endoplasmic reticulum (membrane) with a certainty of 0.1000. The Signal P predicts a likely cleavage site for a SEC12 peptide is between positions 21 and 22, i.e., at the dash in the sequence VRG-AP.

Table 12B. SEC12 protein sequence (SEQ ID NO:24)
MFLSILVALCLWLHLALGVRGAPCEAVRIPMCRHMPWNITRMPNHLHHSTQENAILAIEQ YEELVDVNCSAVLRFFFCAMYAPICTLEFLHDPIKPCKSVCQRARDDCEPLMKMYNHSWP ESLACDELPVYDRGVCISPEAIVTDLPEDVKWIDITPDMMVQERPLDVDCKRLSPDRCKC KKVKPTLATYLSKNYSYVIHAKIKAVQRSGCNEVTTVVDVKEIFKSSSPIPRTQVPLITN SSCQCPHILPHQDVLIMCYEWRSRMMLLENCLVEKWRDQLSKRSIQWEERLQEQRRTVQD KKKTAGRTSRSNPPKPKGKPPAPKPASPKKNIKTRSAQKRTNPKRV

A BLAST analysis of SEC12 was run against the proprietary PatP GENESEQ Protein
Patent database. It was found, for example, that the amino acid sequence of SEC12 had high
5   homology to other proteins as shown in Table 12C.

Table 12C. BLASTX results from PatP database for SEC12		
Sequences producing High-scoring Segment Pairs:	High Score	Smallest Sum Probability P(N)
patp:AAB00193 Breast cancer protein BCX2 - Homo sapiens	1879	9.5e-194
patp:AAB76853 Human lung tumour protein related protein	1879	9.5e-194
patp:AAW73508 Human ATG-1639 protein	1870	8.6e-193
patp:AAY03232 Full length sequence of the human frezzled	1865	2.9e-192
patp:AAB48183 Human FRAZZLED polypeptide	1865	2.9e-192

In a search of public sequence databases, it was found, for example, that the amino
acid sequence of the SEC12 protein of the present invention was found to have 346 of 346
10   amino acid residues (100%) identical to the 346 amino acid NM_003014.   SEC12 also has
homology to the other proteins shown in the BLASTP data in Table 12D.

Table 12D. SEC12 BLASTP results					
Gene Index / Identifier	Protein / Organism	Length (aa)	Identity (%)	Positive (%)	Expect
gi|4506895|re f|NP_003005.1 | (NM_003014)	secreted frizzled- related protein 4; secreted frizzled-related protein 4 [Homo sapiens]	346	346/346 (100)	346/346 (100)	e-179
gi|14749431|r ef|XP_004706. 3| (XM_004706)	secreted frizzled- related protein 4; secreted frizzled-related protein 4 [Homo sapiens]	346	345/346 (99)	345/346 (99)	e-178

gi|7672423|gb |AAF66480.1|A F140346_1 (AF140346)	frizzled related protein [Rattus norvegicus]	348	319/345 (92)	327/345 (94)	e-173
gi|16758312|r ef|NP_445996. 1| (NM_053544)	frizzled related protein 4 [Rattus norvegicus]	348	318/345 (92)	327/345 (94)	e-171
gi|7710094|re f|NP_057896.1 | (NM_016687)	frizzled related protein sequence 4 [Rattus norvegicus]	351	322/348 (92)	331/348 (94)	e-168

This BLASTP data is displayed graphically in the ClustalW in Table 12E. A multiple sequence alignment is given in Table 12E, with the SEC12 protein being shown on line 1, in a ClustalW analysis comparing the protein of the invention with the related protein sequences shown in Table 12D.

Table 12E. ClustalW

```
        1)NOV12      (SEQ ID NO:24)
        2)gi|4506895|    (SEQ ID NO:96)
        3)gi|14749431|   (SEQ ID NO:97)
        4)gi|7672423|    (SEQ ID NO:98)
        5)gi|16758312|   (SEQ ID NO:99)
        6)gi|7710094|    (SEQ ID NO:100)

                           10        20        30        40        50
                      ....|....|....|....|....|....|....|....|....|....|
        SEC12         MFLSILVALCLWLHLALGVRGAPCEAVRIPMCRHMPWNITRMPNHLHHST
        gi|4506895|   MFLSILVALCLWLHLALGVRGAPCEAVRIPMCRHMPWNITRMPNHLHHST
        gi|14749431|  MFLSILVALCLWLHLALGVRGAPCEAVRIPMCRHMPWNITRMPNHLHHST
        gi|7672423|   MLLSILVALCLWLRLALGVRGAPCEAVRIPMCRHMPWNITRMPNHLHHST
        gi|16758312|  MLLSILVALCLCVRLALGVRGAPCEAVRIPMCRHMPWNITRMPNHLHHST
        gi|7710094|   MLRSILVALCLWLRLALGVRGAPCEAVRIPMCRHMPWNITRMPNHLHHST

                           60        70        80        90        100
                      ....|....|....|....|....|....|....|....|....|....|
        SEC12         QENAILAIEQYEELVDVNCSAVLRFFFCAMYAPICTLEFLHDPIKPCKSV
        gi|4506895|   QENAILAIEQYEELVDVNCSAVLRFFFCAMYAPICTLEFLHDPIKPCKSV
        gi|14749431|  QENAILAIEQYEELVDVNCSAVLRFFLCAMYAPICTLEFLHDPIKPCKSV
        gi|7672423|   QENAILAIGQYEELVDVNCSSVLSFFLCAMYAPICTLEFLHDPIKPCKSV
        gi|16758312|  QENAILAIEQYEELVDVNCSSVLRFFLCAMYAPICTLEFLHDPIKPCKSV
        gi|7710094|   QENAILAIEQYEELVDVNCSSVLRFFLCAMYAPICTLEFLHDPIKPCKSV

                           110       120       130       140       150
                      ....|....|....|....|....|....|....|....|....|....|
        SEC12         CQRARDDCEPLMKMYNHSWPESLACDELPVYDRGVCISPEAIVTDLPEDV
        gi|4506895|   CQRARDDCEPLMKMYNHSWPESLACDELPVYDRGVCISPEAIVTDLPEDV
        gi|14749431|  CQRARDDCEPLMKMYNHSWPESLACDELPVYDRGVCISPEAIVTDLPEDV
        gi|7672423|   CQRARDDCEPLMKMYNHSWPESLACDELPVYDRGVCISPEAIVTDLPEDV
        gi|16758312|  CQRARDDCEPLMKMYNHSWPESLACDELPVYDRGVCISPEAIVTDLPEDV
        gi|7710094|   CQRARDDCEPLMKMYNHSWPESLACDELPVYDRGVCISPEAIVTDLPEDV

                           160       170       180       190       200
                      ....|....|....|....|....|....|....|....|....|....|
        SEC12         KWIDITPDMMVQERPLDVDCKRLSPDRCKCKKVKPTLATYLSKNYSYVIH
        gi|4506895|   KWIDITPDMMVQERPLDVDCKRLSPDRCKCKKVKPTLATYLSKNYSYVIH
        gi|14749431|  KWIDITPDMMVQERPLDVDCKRLSPDRCKCKKVKPTLATYLSKNYSYVIH
        gi|7672423|   KWIDITPDMMVQERSFDADCKHLSPDRCKCKKVKPTLATYLSKNYSYVIH
        gi|16758312|  KWIDITPDMMVQERSFDADCKHLSPDRCKCKKVKPTLATYLSKNYSYVIH
        gi|7710094|   KWIDITPDMMVQERSFDADCKRLSPDRCKCKKVKPTLATYLSKNYSYVIH
```

```
                            210       220       230       240       250
                    ....|....|....|....|....|....|....|....|....|....|
    SEC12           AKIKAVQRSGCNEVTTVVDVKEIFKSSSPIPRTQVPLITNSSCQCPHILP
    gi|4506895|     AKIKAVQRSGCNEVTTVVDVKEIFKSSSPIPRTQVPLITNSSCQCPHILP
    gi|14749431|    AKIKAVQRSGCNEVTTVVDVKEIFKSSSPIPRTQVPLITNSSCQCPHILP
    gi|7672423|     AKIKAVQRSGCNEVTTVVDVKEIFKSSSPIPRTQVPLITNSSCQCPHILP
    gi|16758312|    AKIKAVQRSGCNEVTTVVDVKEIFKSSSPIPRTQVPLITNSSCQCPHILP
    gi|7710094|     AKIKAVQRSGCNEVTTVVDVKEIFKSISPIPRTQVPLITNSSCQCPHILP

                            260       270       280       290       300
                    ....|....|....|....|....|....|....|....|....|....|
    SEC12           HQDVLIMCYEWRSRMMLLENCLVEKWRDQLSKRSIQWEERLQEQRRTVQD
    gi|4506895|     HQDVLIMCYEWRSRMMLLENCLVEKWRDQLSKRSIQWEERLQEQRRTVQD
    gi|14749431|    HQDVLIMCYEWRSRMMLLENCLVEKWRDQLSKRSIQWEERLQEQRRTVQD
    gi|7672423|     HQDVLIMCYERRSRMMLLENCLVEKWRDQLSRRSTQWEERLQEQQRTTQD
    gi|16758312|    XQDVLIMCYERRSRMMLLENCLVEKWRDQLSRRSTQWEERLQEQQRTTQD
    gi|7710094|     HQDVLIMCYEWRSRMMLLENCLVEKWRDQLSRRSIQWEERLQEQQRTIQD

                            310       320       330       340       350
                    ....|....|....|....|....|....|....|....|....|....|
    SEC12           KKKTAGRTS---RSNPPKPKGKPPAPKPASPKKNIKTRSAQKRINPKRV-
    gi|4506895|     KKKTAGRTS---RSNPPKPKGKPPAPKPASPKKNIKTRSAQKRINPKRV-
    gi|14749431|    KKKTAGRTS---RSNPPKPKGKPPAPKPASPKKNIKTRSAQKRINPKRV-
    gi|7672423|     KKQIASRTS---RSNPPKPKGRSPASKPASPKKNIKARSAPKKSNPKKST
    gi|16758312|    KKQIASRTS---RSNPPKPKGRSPASKPASPKKNIKARSAPKKSNPKKST
    gi|7710094|     KKQIASRTSRTSRSNPPKSKGRPPAPKPASPKKNIKARSAPKKSNLKKSA


    SEC12           -
    gi|4506895|     -
    gi|14749431|    -
    gi|7672423|     S
    gi|16758312|    S
    gi|7710094|     S
```

The presence of identifiable domains in SEC12, as well as all other SECX proteins, was determined by searches using software algorithms such as PROSITE, DOMAIN, Blocks, Pfam, ProDomain, and Prints, and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (http:www.ebi.ac.uk/ interpro). DOMAIN results for SEC12 as disclosed in Table 12F, were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST analyses. This BLAST analysis software samples domains found in the Smart and Pfam collections. Fully conserved single residues are indicated by the sign (|) and "strong" semi-conserved residues are indicated by the sign (+). The "strong" group of conserved amino acid residues may be any one of the following groups of amino acids: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW.

Table 12F lists the domain description from DOMAIN analysis results against SEC12. This indicates that the SEC12 sequence has properties similar to those of other proteins known to contain this domain.

Table 12F. Domain Analysis of SEC12

gnl|Smart|smart00063, FRI, Frizzled; Drosophila melanogaster frizzled mediates signalling that polarises a precursor cell along the anteroposterior axis. Homologues of the N-terminal region of frizzled exist either as transmembrane or secreted molecules. Frizzled homologues are reported to be receptors for the Wnt growth factors.

CD-Length = 117 residues, 98.3% aligned

Score = 139 bits (350), Expect = 3e-34

```
SEC12:    23    PCEAVRIPMCRHMPWNITRMPNHLHHSTQENAILAIEQYEELVDVNCSAVLRFFFCAMYA   82
                CE + +P+C+ + +N+T MPN L H+TQE A L + Q+  L++V CS  LRFF C++YA
Sbjct:    1     RCEPITLPLCKDLGYNLTSMPNLLGHTTQEEAGLELSQFYPLLNVQCSPDLRFFLCSVYA   60

SEC12:    83    PICTLEFLHDPIKPCKSVCQRARDDCEPLMKMYNHSWPESLACDELPVYDRGVCISP     139
                P+CT E L +PI PC+S+C+ AR+ CEPLM+ +   WPE L CD  PV +  +C+ P
Sbjct:   61     PVCTEDLPEPILPCRSLCEAAREGCEPLMEKFGFGWPEFLRCDRFPVQNELCMDPVP   120
(SEQ ID NO: 306)
```

Frizzled (FZD) genes, disclosed herein as SEC11, encode receptors for WNTs, which play key roles in carcinogenesis and embryogenesis. Homologues of the N-terminal region of frizzled exist either as transmembrane or secreted molecules. The secreted frizzled related protein 2 (sFRP2) is upregulated within 2 days of in vitro development.   In vivo sFRP2

5   expression was likewise found in mesenchymal condensates and subsequent epithelial structures. Detailed in situ hybridization analysis revealed sFRP2 expression during development of the eye, brain, neural tube, craniofacial mesenchyme, joints, testis, pancreas and below the epithelia of oesophagus, aorta and ureter where smooth muscles develop. In a comparative analysis, transcripts of the related sFRP1 and sFRP4 genes were frequently found

10   in the same tissues as sFRP2 with their expression domains overlapping in some instances, but mutually exclusive in others. While sFRP1 is specifically expressed in the embryonic metanephros, eye, brain, teeth, salivary gland and small intestine, there is only weak expression of sFRP4 except for the developing teeth, eye and salivary gland. Nevertheless, sFRP genes play quite distinct roles in the morphogenesis of several organ systems.

15   The SEC12 disclosed in this invention is expressed in at least the following tissues: apical microvilli of the retinal pigment epithelium, arterial (aortic), basal forebrain, brain, Burkitt lymphoma cell lines, corpus callosum, cardiac (atria and ventricle), caudate nucleus, CNS and peripheral tissue, cerebellum, cerebral cortex, colon, cortical neurogenic cells, endothelial (coronary artery and umbilical vein) cells, palate epithelia, eye, neonatal eye,

20   frontal cortex, fetal hematopoietic cells, heart, hippocampus, hypothalamus, leukocytes, liver, fetal liver, lung, lung lymphoma cell lines, fetal lymphoid tissue, adult lymphoid tissue, those that express MHC I, II, and III, nervous tissues, medulla, subthalamic nucleus, ovary, pancreas, pituitary, placenta, pons, prostate, putamen, serum, skeletal muscle, small intestine,

smooth muscle (coronary artery in aortia) spinal cord, spleen, stomach, taste receptor cells of the tongue, testis, thalamus, and thymus tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, public EST sources, literature sources, and/or RACE sources.

5   Further expression data for SEC12 is provided in Example 2.

The nucleic acids and proteins of SEC12 are useful in potential therapeutic applications implicated in various SEC-related pathological disorders described further herein. The SEC12 nucleic acid encoding the frizzled-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of

10   the nucleic acid or the protein are to be assessed.

These materials are further useful in the generation of antibodies that bind immunospecifically to the substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-SECX Antibodies" section

15   below. The disclosed SEC12 protein has multiple hydrophilic regions, each of which can be used as an immunogen. In one embodiment, for example, a contemplated SEC12 epitope comprises from about amino acids 35 to about 65. In another embodiment, for example, a SEC12 epitope comprises from about amino acids 95 to about 135. In further embodiments, for example, a SEC12 epitope comprises from about 145 to about 258, and from about 260 to

20   about 346.

NOVX

The present invention also provides novel nucleotides and polypeptides encoded thereby. Included in the invention are the novel nucleic acid sequences and their encoded polypeptides. The sequences are collectively referred to herein as "NOVX nucleic acids" or

25   "NOVX polynucleotides" and the corresponding encoded polypeptides are referred to as "NOVX polypeptides" or "NOVX proteins." Unless indicated otherwise, "NOVX" is meant to refer to any of the novel sequences disclosed herein. Table A provides a summary of the NOVX nucleic acids and their encoded polypeptides.

TABLE 12G. Sequences and Corresponding SEQ ID Numbers

30

NOVX Assignment	Internal Identification	SEQ ID NO (nucleic acid)	SEQ ID NO (polypeptide)	Homology
1	CG56008	25	26	LIV-1
2	CG56149	27	38	NRD convertase
3	CG56155	29	30	Kallikrein
4	CG56166	31	32	Multidrug transporter

5	CG56151	33	34	Glucose transporter type 2
6	CG56690	35	36	Frizzled homolog 9
7	CG55117	37	38	AC133, prominin
8	CG56006	39	40	hepsin

NOVX nucleic acids and their encoded polypeptides are useful in a variety of
applications and contexts. The various NOVX nucleic acids and polypeptides according to the
invention are useful as novel members of the protein families according to the presence of

5   domains and sequence relatedness to previously described proteins. Additionally, NOVX
nucleic acids and polypeptides can also be used to identify proteins that are members of the
family to which the NOVX polypeptides belong.

NOV1 is homologous to a NRD convertase-like family of proteins. Thus, the NOV1
nucleic acids, polypeptides, antibodies and related compounds according to the invention will

10   be useful in therapeutic and diagnostic applications implicated in, for example; cancer,
especially breast, ovarian and bladder cancer, and/or other pathologies or conditions.

NOV2 is homologous to the NRD convertase-like family of proteins. Thus NOV2
nucleic acids, polypeptides, antibodies and related compounds according to the invention will
be useful in therapeutic and diagnostic applications implicated in diabetes, metabolic disorders

15   and/or other pathologies and disorders.

NOV3 is homologous to a family of kallikrein-like proteins. Thus, the NOV3 nucleic
acids and polypeptides, antibodies and related compounds according to the invention will be
useful in therapeutic and diagnostic applications implicated in, for example: cancer,
particularly prostate cancer, metabolic disorders, heart disease, hypertension, and/or other

20   pathologies.

NOV4 is homologous to the multidrug transporter-like family of proteins. Thus, NOV4
nucleic acids, polypeptides, antibodies and related compounds according to the invention will
be useful in therapeutic and diagnostic applications implicated in, for example: cancer,
especially leukemia, metabolic disorders, and/or other pathologies.

25   NOV5 is homologous to the glucose transporter type 2-like family of proteins. Thus
NOV5 nucleic acids, polypeptides, antibodies and related compounds according to the
invention will be useful in therapeutic and diagnostic applications implicated in diabetes, fetal
growth retardation, cancer, glycogen storage disease, hypertension and/or other disorders and
conditions.

30   NOV6 is homologous to the Frizzled 9-like family of proteins. Thus NOV6 nucleic
acids, polypeptides, antibodies and related compounds according to the invention will be

useful in therapeutic and diagnostic applications implicated in, for example: ulcerative colitis, Crohn's disease, recessive Robinow syndrome, cancer and/or other pathologies/disorders.

NOV7 is homologous to members of the prominin-like family of proteins. Thus, the NOV7 nucleic acids, polypeptides, antibodies and related compounds according to the
5   invention will be useful in therapeutic and diagnostic applications implicated in, for example; neurological disorders, cholesterol transport disorders, retinal degeneration and/or other pathologies/disorders.

NOV8 is homologous to the hepsin-like family of proteins. Thus, NOV8 nucleic acids and polypeptides, antibodies and related compounds according to the invention will be useful
10   in therapeutic and diagnostic applications implicated in, for example; cancer, especially prostate and ovarian cancer, and/or other pathologies/disorders.

The NOVX nucleic acids and polypeptides can also be used to screen for molecules, which inhibit or enhance NOVX activity or function. Specifically, the nucleic acids and polypeptides according to the invention may be used as targets for the identification of small
15   molecules that modulate or inhibit, e.g., neurogenesis, cell differentiation, cell proliferation, hematopoiesis, wound healing and angiogenesis.

Additional utilities for the NOVX nucleic acids and polypeptides according to the invention are disclosed herein.

NOV1

20   A disclosed NOV1 nucleic acid of 3445 nucleotides designated SEQ ID NO:25 (also referred to as CG56008) encoding a human LIV-1-like protein is shown in Table 13A.

Table 13A. NOV1 nucleotide sequence (SEQ ID NO:25).
CACCGCGTGTTCGCGCCTGGTAGAGATTTCTCGAAGACACCAGTGGGCCCGTGTGGAACCAAACCTGCGCGCGTGGCCGG
GCCGTGGGACAACGAGGCCGCGGAGACGAAGGCGCAATGGCGAGGAAGTTATCTGTAATCTTGATCCTGACCTTTGCCCT
CTCTGTCACAAATCCCCTTCATGAACTAAAAGCAGCTGCTTTCCCCCAGACCACTGAGAAAATTAGTCCGAATTGGGAAT
CTGGCATTAATGTTGACTTGGCAATTTCCACACGGCAATATCATCTACAACAGCTTTTCTACCGCTATGGAGAAAATAAT
TCTTTGTCAGTTGAAGGGTTCAGAAAATTACTTCAAAATATAGGCATAGATAAGATTAAAAGAATCCATATACACCATGA
CCACGACCATCACTCAGACCACGAGCATCACTCAGACCATGAGCGTCACTCAGACCATGAGCATCACTCAGAGCACGAGC
ATCACTCTGACCATGATCATCACTCTCACCATAATCATGCTGCTTCTGGTAAAAATAAGCGAAAGCTCTTTGCCCAGAC
CATGACTCAGATAGTTCAGGTAAAGATCCTAGAAACAGCCAGGGGAAAGGAGCTCACCGACCAGAACATGCCAGTGGTAG
AAGGAATGTCAAGGACAGTGTTAGTGCTAGTGAAGTGACCTCAACTGTGTACAACACTGTCTCTGAAGGAACTCACTTTC
TAGAGACAATAGAGACTCCAAGACCTGGAAAACTCTTCCCCAAAGATGTAAGCAGCTCCACTCCACCCAGTGTCACATCA
AAGAGCCGGGTGAGCCGGCTGGCTGGTAGGAAAACAAATGAATCTGTGAGTGAGCCCCGAAAAGGCTTTATGTATTCCAG
AAACACAAATGAAAATCCTCAGGAGTGTTTCAATGCATCAAAGCTACTGACATCTCATGGCATGGGCATCCAGGTTCCGC
TGAATGCAACAGAGTTCAACTATCTCTGTCCAGCCATCATCAACCAAATTGATGCTAGATCTTGTCTGATTCATACAAGT
GAAAAGAAGGCTGAAATCCCTCCAAAGACCTATTCATTACAAATAGCCTGGGTTGGTGGTTTTATAGCCATTTCCATCAT
CAGTTTCCTGTCTCTGCTGGGGGTTATCTTAGTGCCTCTCATGAATCGGGTGTTTTTCAAATTTCTCCTGAGTTTCCTTG
TGGCACTGGCCGTTGGGACTTTGAGTGGTGATGCTTTTTTACACCTTCTTCCACATTCTCATGCAAGTCACCACCATAGT
CATAGCCATGAAGAACCAGCAATGGAAATGAAAAGAGGACCACTTTTCAGTCATCTGTCTTCTCAAAACATAGAAGAAAG
TGCCTATTTTGATTCCACGTGGAAGGGTCTAACAGCTCTAGGAGGCCTGTATTTCATGTTTCTTGTTGAACATGTCCTCA
CATTGATCAAACAATTTAAAGATAAGAAGAAAAAGAATCAGAAGAAACCTGAAAATGATGATGATGTGGAGATTAAGAAG
CAGTTGTCCAAGTATGAATCTCAACTTTCAACAAATGAGGAGAAAGTAGATACAGATGATCGAACTGAAGGCTATTTACG
AGCAGACTCACAAGAGCCCTCCCACTTTGATTCTCAGCAGCCTGCAGTCTTGGAAGAAGAAGAGGTCATGATAGCTCATG
CTCATCCACAGGAAGTCTACAATGAATATGTACCCAGAGGGTGCAAGAATAAATGCCATTCACATTTCCACGATACACTC
GGCCAGTCAGACGATCTCATTCACCACCATCATGACTACCATCATATTCTCCATCATCACCACCAAAACCACCATCC
TCACAGTCACAGCCAGCGCTACTCTCGGGAGGAGCTGAAAGATGCCGGCGTCGCCACTCTGGCCTGGATGGTGATAATGG
GTGATGGCCTGCACAATTTCAGCGATGGCCTAGCAATTGGTGCTGCTTTTACTGAAGGCTTATCAAGTGGTTTAAGTACT

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TCTGTTGCTGTGTTCTGTCATGAGTTGCCTCATGAATTAGGTGACTTTGCTGTTCTACTAAAGGCTGGCATGACCGTTAA
GCAGGCTGTCCTTTATAATGCATTGTCAGCCATGCTGGCGTATCTTGGAATGGCAACAGGAATTTTCATTGGTCATTATG
CTGAAAATGTTTCTATGTGGATATTTGCACTTACTGCTGGCTTATTCATGTATGTTGCTCTGGTTGATATGGTACCTGAA
ATGCTGCACAATGATGCTAGTGACCATGGATGTAGCCGCTGGGGGTATTTCTTTTTACAGAATGCTGGGATGCTTTTGGG
TTTTGGAATTATGTTACTTATTTCCATATTTGAACATAAAATCGTGTTTCGTATAAATTTCTAGTTAAGGTTTAAATGCT
AGAGTAGCTTAAAAAGTTGTCATAGTTTCAGTAGGTCATAGGGAGATGAGTTTGTATGCTGTACTATGCAGCGTTTAAAG
TTAGTGGGTTTTGTGATTTTTGTATTGAATATTGCTGTCTGTTACAAAGTCAGTTAAAGGTACGTTTTAATATTTAAGTT
ATTCTATCTTGGAGATAAAATCTGTATGTGCAATTCACCGGTATTACCAGTTTATTATGTAAACAAGAGATTTGGCATGA
CATGTTCTGTATGTTTCAGGGAAAAATGTCTTTAATGCTTTTTCAAGAACTAACACAGTTATTCCTATACTGGATTTTAG
GTCTCTGAAGAACTGCTGGTGTTTAGGAATAAGAATGTGCATGAAGCCTAAAATACCAAGAAAGCTTATACTGAATTTAA
GCAAAGAAATAAAGGAGAAAAGAGAAGAATCTGAGAATTGGGGAGGCATAGATTCTTATAAAAATCACAAAATTTGTTGT
AAATTAGAGGGGAGAAATTTAGAATTAAGTATAAAAAGGCAGAATTAGTATAGAGTACATTCATTAAACATTTTTGTCAG
GATTATTTCCCGTAAAAACGTAGTGAGCACTTTTCATATACTAATTTAGTTGTACATTTAACTTTGTATAATACAGAAAT
CTAAATATATTTAATGAATTCAAGCAATATATCACTTGACCAAGAAATTGAATTTCAAAATGTTCGTGCGGGTATATAC
CAGATGAGTACAGTGAGTAGTTTTATGTATCACCAGACTGGGTTATTGCCAAGTTATATATCACCAAAAGCTGTATGACT
GGATGTTCTGGTTACCTGGTTTACAAAATTATCAGAGTAGTAAAACTTTGATATATATGAGGATATTAAAACTACACTAA
GTATCATTTGATTCGATTCAGAAAGTACTTTGATATCTCTCAGTGCTTCAGTGCTATCATTGTGAGCAATTGTCTTTTAT
ATACGGTACTGTAGCCATACTAGGCCTGTCTGTGGCATTCTCTAGATGTTTCTTTTTTACACAATAAATTCCTTATATCA
GCTTG
```

The disclosed NOV1 polypeptide (SEQ ID NO:26) encoded by SEQ ID NO:25 has
755 amino acid residues and is presented in Table 13A using the one-letter amino acid code.
Signal P, Psort and/or Hydropathy results predict that NOV1 contains a signal peptide and is
5   likely to be localized to the plasma membrane with a certainty of 0.6400. In other
embodiments, NOV1 may also be localized to the Golgi body with a certainty of 0.4600, the
endoplasmic reticulum (membrane) with a certainty of 0.3700, or the endoplasmic reticulum
(lumen) with a certainty of 0.1000. The most likely cleavage site for NOV1 is between
positions 18 and 19: SVT-NP.

10

Table 13B. Encoded NOV1 protein sequence (SEQ ID NO:26).
MARKLSVILILTFALSVTNPLHELKAAAFPQTTEKISPNWESGINVDLAISTRQYHLQQLFYRYGENNSLSVEGFRKLLQ NIGIDKIKRIHIHHDHDHHSDHEHHSDHERHSDHEHHSEHEHHSDHDHHSHHNHAASGKNKRKALCPDHDSDSSGKDPRN SQGKGAHRPEHASGRRNVKDSVSASEVTSTVYNTVSEGTHFLETIETPRPGKLFPKDVSSSTPPSVTSKSRVSRLAGRKT NESVSEPRKGFMYSRNTNENPQECFNASKLLTSHGMGIQVPLNATEFNYLCPAIINQIDARSCLIHTSEKKAEIPPKTYS LQIAWVGGFIAISIISFLSLLGVILVPLMNRVFFKFLLSFLVALAVGTLSGDAFLHLLPHSHASHHHSHSHEEPAMEMKR GPLFSHLSSQNIEESAYFDSTWKGLTALGGLYFMFLVEHVLTLIKQFKDKKKKNQKKPENDDDVEIKKQLSKYESQLSTN EEKVDTDDRTEGYLRADSQEPSHFDSQQPAVLEEEEVMIAHAHPQEVYNEYVPRGCKNKCHSHFHDTLGQSDDLIHHHHD YHHILHHHHQNHHPHSHSQRYSREELKDAGVATLAWMVIMGDGLHNFSDGLAIGAAFTEGLSSGLSTSVAVFCHELPHE LGDFAVLLKAGMTVKQAVLYNALSAMLAYLGMATGIFIGHYAENVSMWIFALTAGLFMYVALVDMVPEMLHNDASDHGCS RWGYFFLQNAGMLLGFGIMLLISIFEHKIVFRINF

NOV1 is expressed in at least the following tissues: Adrenal gland, Aorta, Blood,
Bone, Brain, Breast, Colon, Ear, Foreskin, Kidney, Lung, Ovary, Parathyroid,Placenta,
Pooled, Prostate, Stomach, Testis, Thyroid, Tonsil, Uterus, Whole embryo, amnion_normal,
15   breast, breast_normal, colon, head_neck, kidney, lung, marrow, nervous_normal,
nervous_tumor, ovary,placenta, placenta_normal, prostate_tumor, skin, testis_normal, uterus,
uterus_tumor. This information was derived by determining the tissue sources of the
sequences that were included in the invention including but not limited to SeqCalling sources,
Public EST sources, Literature sources, and/or RACE sources.

20

The disclosed NOV1 polypeptide has homology to the amino acid sequences shown in the BLASTP data listed in Table 13C. Public amino acid databases include the GenBank databases, SwissProt, PDB and PIR.

Table 13C. BLAST results for NOV1					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Po sitives (%)	Expect
gi|7513131|pir||G02 273	LIV-1 protein - human	752	98	98	0.0
gi|14760892|ref|XP_ 029402.1| (XM_029402)	LIV-1 protein, estrogen regulated [Homo sapiens]	755	100	100	0.0
gi|12751475|ref|NP_ 036451.2| (NM_012319)	LIV-1 protein, estrogen regulated [Homo sapiens]	749	98	98	0.0
gi|14249879|gb|AAH0 8317.1|AAH08317 (BC008317)	Unknown (protein for IMAGE:3343159) [Homo sapiens]	382	100	100	0.0
gi|505102|dbj|BAA06 685.1| (D31887)	KIAA0062 [Homo sapiens]	531	29	46	1e-55

5

The homology between these and other sequences is shown graphically in the ClustalW analysis shown in Table 13D. In the ClustalW alignment of the NOV1 proteins, as well as all other ClustalW analyses herein, the black outlined amino acid residues indicate regions of conserved sequence (*i.e.*, regions that may be required to preserve structural or

10   functional properties), whereas non-highlighted amino acid residues are less conserved and can potentially be altered to a much broader extent without altering protein structure or function.

Table 13D. ClustalW Analysis of NOV1

15
```
    1)   Novel NOV1   (SEQ ID NO:25)
    2)   gi|7513131|    (SEQ ID NO:101)
    3)   gi|14760892|   (SEQ ID NO:102)
    4)   gi|12751475|   (SEQ ID NO:103)
    5)   gi|14249879|   (SEQ ID NO:104)
20  6)   gi|505102|    (SEQ ID NO:105)

                           10        20        30        40        50
                     ....|....|....|....|....|....|....|....|....|....|....|
         NOV1        MARKLSVILILTFALSVTNPLHELKAAAFPQTTEKISPNWESGINVDLAI
25       gi|7513131| MARKLSVILILTFALSVTNPLHELKAAAFPQTTEKISPNWESGINVDLAI
         gi|14760892| MARKLSVILILTFALSVTNPLHELKAAAFPQTTEKISPNWESGINVDLAI
         gi|12751475| MARKLSVILILTFALSVTNPLHELKAAAFPQTTEKISPNWESGINVDLAI
         gi|14249879| -------------------------------------------------
         gi|505102|  ----------------------RVXADAPAKXLLPPPXAWDXAXRX
30
                               60        70        80        90       100
```

```
                    . . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|
        NOV1        STRQYHLQQLFYRYGENNSLSVEGFRKLLQNIGIDKIKRIHIHHDHDHHS
        gi|7513131| STRQYHLQQLFYRYGENNSLSVEGFRKLLQNIGIDKIKRIHIHHDHDHHS
        gi|14760892|STRQYHLQQLFYRYGENNSLSVEGFRKLLQNIGIDKIKRIHIHHDHDHHS
        gi|12751475|STRQYHLQQLFYRYGENNSLSVEGFRKLLQNIGIDKIKRIHIHHDHDHHS
        gi|14249879|-------------------------------------------------
        gi|505102|  RGAEAASERQVYSVTMKLLLLHPAFOSCLLLTLEGLWR-----------

                         110       120       130       140       150
                    . . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|
        NOV1        DHEHHSDHERHSDHEHHSEHEHHSDHDHHSHHNHAASGKNKRKALCPDHD
        gi|7513131| DHEHHSDHERHSDHEHHSDHEHHSDHN-----HAASGKNKRKALCPDHD
        gi|14760892|DHEHHSDHERHSDHEHHSEHEHHSDHDHHSHHNHAASGKNKRKALCPDHD
        gi|12751475|DHEHHSDHERHSDHEHHSDHEHHSDHN-----HAASGKNKRKALCPDHD
        gi|14249879|-------------------------------------------------
        gi|505102|  ----------------TIPEAHASSLG----------------------

                         160       170       180       190       200
                    . . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|
        NOV1        SDSSGKDPRNSQGKGAHRPEHASGRRNVKDSVSASEVTSTVYNTVSEGTH
        gi|7513131| SDSSGKDPRNSQGKGAHRPEHASGRRNVKDSVSASEVTSTVYNTVSEGTH
        gi|14760892|SDSSGKDPRNSQGKGAHRPEHASGRRNVKDSVSASEVTSTVYNTVSEGTH
        gi|12751475|SDSSGKDPRNSQGKGAHRPEHASGRRNVKDSVSASEVTSTVYNTVSEGTH
        gi|14249879|-------------------------------------------------
        gi|505102|  --------------------------APAISAASFLQDDIHRYGE---

                         210       220       230       240       250
                    . . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|
        NOV1        FLETIETPRPGKLFPKDVSSSTPPSVTSKSRVSRLAGRKTNESVSEPRKG
        gi|7513131| FLETIETPRPGKLFPKDVSSSTPPSVTSKSRVSRLAGRKTNESVSEPRKG
        gi|14760892|FLETIETPRPGKLFPKDVSSSTPPSVTSKSRVSRLAGRKTNESVSEPRKG
        gi|12751475|FLETIETPRPGKLFPKDVSSSTPPSVTSKSRVSRLAGRKTNESVSEPRKG
        gi|14249879|-------------------------------------------------
        gi|505102|  ----------G--------------DS--LTIQQLKALLNHLDVGVGRGN

                         260       270       280       290       300
                    . . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|
        NOV1        FMYSRNTNENPQECFNASKLLTSHGMGIQVPLNATEFNYLCPAIINQIDA
        gi|7513131| FMYSRNTNENPQECFNASKLLTSHGMGIQVPLNATEFNYLCPAIINQIDA
        gi|14760892|FMYSRNTNENPQECFNASKLLTSHGMGIQVPLNATEFNYLCPAIINQIDA
        gi|12751475|FMYSRNTNENPQECFNASKLLTSHGMGIQVPLNATEFNYLCPAIINQIDA
        gi|14249879|-------------------------------------------------
        gi|505102|  VTQHVQGHRNLSTCFSSGDLFTAINFSEQSRIGSSELQEFCPTILQQLDS

                         310       320       330       340       350
                    . . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|
        NOV1        RSCLIHT--SEKKAEIPPKTYSLQIAWVGGFIAISIISFLSLLGVILVPL
        gi|7513131| RSCLIHT--SEKKAEIPPKTYSLQIAWVGGFIAISIISFLSLLGVILVPL
        gi|14760892|RSCLIHT--SEKKAEIPPKTYSLQIAWVGGFIAISIISFLSLLGVILVPL
        gi|12751475|RSCLIHT--SEKKAEIPPKTYSLQIAWVGGFIAISIISFLSLLGVILVPL
        gi|14249879|-------------------------------------------------
        gi|505102|  RACTSENQENEENEQTEEGRPSAVEVWGYGLICVEVISLCSLLGASVVPF

                         360       370       380       390       400
                    . . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|
        NOV1        MNRVFFKFLLSFLVALAVGTLSGDAFLHLLPHSHASHHHSHSHEEPAMEM
        gi|7513131| MNRVFFKFLLSFLVALAVGTLSGDAFLHLLPHSHASHHHSHSHEEPAMEM
        gi|14760892|MNRVFFKFLLSFLVALAVGTLSGDAFLHLLPHSHASHHHSHSHEEPAMEM
        gi|12751475|MNRVFFKFLLSFLVALAVGTLSGDAFLHLLPHSHASHHHSHSHEEPAMEM
        gi|14249879|------------------------FLHLLPHSHASHHHSHSHEEPAMEM
        gi|505102|  MKKTFYKRLLLXFIALAIGTLYSNALFQLIPEAFG--------------

                         410       420       430       440       450
                    . . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|
        NOV1        KRGPLFSHLSSQNIEESAYFDSTWKGLTALGGLYFMFLVEHVLTLIKQFK
        gi|7513131| KRGPLFSHLSSQNIEESAYFDSTWKGLTALGGLYFMFLVEHVLTLIKQFK
        gi|14760892|KRGPLFSHLSSQNIEESAYFDSTWKGLTALGGLYFMFLVEHVLTLIKQFK
        gi|12751475|KRGPLFSHLSSQNIEESAYFDSTWKGLTALGGLYFMFLVEHVLTLIKQFK
        gi|14249879|KRGPLFSHLSSQNIEESAYFDSTWKGLTALGGLYFMFLVEHVLTLIKQFK
        gi|505102|  -FNPLEDXYVS------------KSAVVFGGFYLFFFTEKILKELLKQK

                         460       470       480       490       500
                    . . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|. . . .|
        NOV1        DKKKKNQKKPENDDDVEIKKQLSKYESQLSTNEEKVDTDDRTEGYLRADS
        gi|7513131| DKKKKNQKKPENDDDVEIKKQLSKYESQLSTNEEKVDTDDRTEGYLRADS
        gi|14760892|DKKKKNQKKPENDDDVEIKKQLSKYESQLSTNEEKVDTDDRTEGYLRADS
        gi|12751475|DKKKKNQKKPENDDDVEIKKQLSKYESQLSTNEEKVDTDDRTEGYLRADS
        gi|14249879|DKKKKNQKKPENDDDVEIKKQLSKYESQLSTNEEKVDTDDRTEGYLRADS
```

```
gi|505102|     NEHHHG----------------------------------------------

                        510       520       530       540       550
                   ....|....|....|....|....|....|....|....|....|....|
NOV1           QEPSHFDSQQPAVLEEEEVMIAHAHPQEVYNEYVPRGCKNKCHSHFHDTL
gi|7513131|    QEPSHFDSQQPAVLEEEEVMIAHAHPQEVYNEYVPRGCKNKCHSHFHDTL
gi|14760892|   QEPSHFDSQQPAVLEEEEVMIAHAHPQEVYNEYVPRGCKNKCHSHFHDTL
gi|12751475|   QEPSHFDSQQPAVLEEEEVMIAHAHPQEVYNEYVPRGCKNKCHSHFHDTL
gi|14249879|   QEPSHFDSQQPAVLEEEEVMIAHAHPQEVYNEYVPRGCKNKCHSHFHDTL
gi|505102|     --HSHMASESLPSKKDQEEGVMEKLQNGDLDHMIP----QHCSSELDGKA

                        560       570       580       590       600
                   ....|....|....|....|....|....|....|....|....|....|
NOV1           GQSDDLIHHHHDYHHILHHHHHQNHHPHSHSQRYSREELKDAGVATLAWM
gi|7513131|    GQSDDLIHHHHDYHHILHHHHHQNHHPHSHSQRYSREELKDAGVATLAWM
gi|14760892|   GQSDDLIHHHHDYHHILHHHHHQNHHPHSHSQRYSREELKDAGVATLAWM
gi|12751475|   GQSDDLIHHHHDYHHILHHHHHQNHHPHSHSQRYSREELKDAGVATLAWM
gi|14249879|   GQSDDLIHHHHDYHHILHHHHHQNHHPHSHSQRYSREELKDAGVATLAWM
gi|505102|     PMVD--------EKVIVGSLSVQDLQASQSACYWLKGVRYSDICTLAWM

                        610       620       630       640       650
                   ....|....|....|....|....|....|....|....|....|....|
NOV1           VIMGDGLHNFSDGLAIGAAFTEGLSSGLSTSVAVFCHELPHELGDFAVLL
gi|7513131|    VIMGDGLHNFSDGLAIGAAFTEGLSSGLSTSVAVFCHELPHELGDFAVLL
gi|14760892|   VIMGDGLHNFSDGLAIGAAFTEGLSSGLSTSVAVFCHELPHELGDFAVLL
gi|12751475|   VIMGDGLHNFSDGLAIGAAFTEGLSSGLSTSVAVFCHELPHELGDFAVLL
gi|14249879|   VIMGDGLHNFSDGLAIGAAFTEGLSSGLSTSVAVFCHELPHELGDFAVLL
gi|505102|     FTISDGLHNFIDGLAIGASFTVSMFQGISTSVAILCEEFPHELGDFVLLL

                        660       670       680       690       700
                   ....|....|....|....|....|....|....|....|....|....|
NOV1           KAGMTVKQAVLYNALSAMLAYLGMATGIFIGHYAENVSMWIFALTAGLFM
gi|7513131|    KAGMTVKQAVLYNALSAMLAYLGMATGIFIGHYAENVSMWIFALTAGLFM
gi|14760892|   KAGMTVKQAVLYNALSAMLAYLGMATGIFIGHYAENVSMWIFALTAGLFM
gi|12751475|   KAGMTVKQAVLYNALSAMLAYLGMATGIFIGHYAENVSMWIFALTAGLFM
gi|14249879|   KAGMTVKQAVLYNALSAMLAYLGMATGIFIGHYAENVSMWIFALTAGLFM
gi|505102|     NAGMSTQQAIFENFLSACCCYLGIAFGILAGSHFS--ANWIFALAGGMFI

                        710       720       730       740       750
                   ....|....|....|....|....|....|....|....|....|....|
NOV1           YVALVDMVPEMLHNDASDHG-CSRWGYFFLQNAGMLLGFGIMLLISIFEH
gi|7513131|    YVALVDMVPEMLHNDASDHG-CSRWGYFFLQNAGMLLGFGIMLLIPYLNI
gi|14760892|   YVALVDMVPEMLHNDASDHG-CSRWGYFFLQNAGMLLGFGIMLLISIFEH
gi|12751475|   YVALVDMVPEMLHNDASDHG-CSRWGYFFLQNAGMLLGFGIMLLISIFEH
gi|14249879|   YVALVDMVPEMLHNDASDHG-CSRWGYFFLQNAGMLLGFGIMLLISIFEH
gi|505102|     YISLADMFPEMNEVCQEDERKGSILIPFIIQNLGILTGFTIMVVIMYSG

                        760
                   ....|....|.
NOV1           K---IVFRINF
gi|7513131|    KSCSYKFLQKV
gi|14760892|   K---IVFRINF
gi|12751475|   K---IVFRINF
gi|14249879|   K---IVFRINF
gi|505102|     Q-----IQIG-
```

The presence of identifiable domains in NOV1, as well as all other NOVX proteins, was determined by searches using software algorithms such as PROSITE, DOMAIN, Blocks, Pfam, ProDomain, and Prints, and then determining the Interpro number by crossing the domain match (or numbers) using the Interpro website (http:www.ebi.ac.uk/ interpro). DOMAIN results for NOV1 as disclosed in Tables 13K-13L, were collected from the Conserved Domain Database (CDD) with Reverse Position Specific BLAST analyses. This BLAST analysis software samples domains found in the Smart and Pfam collections. For Table 1K and all successive DOMAIN sequence alignments, fully conserved single residues are indicated by black shading or by the sign (|) and "strong" semi-conserved residues are

indicated by grey shading or by the sign (+). The "strong" group of conserved amino acid

residues may be any one of the following groups of amino acids: STA, NEQK, NHQK,

NDEQ, QHRK, MILV, MILF, HY, FYW.

Tables 1E-F list the domain descriptions from DOMAIN analysis results against

5   NOV1. This indicates that the NOV1 sequence has properties similar to those of other

proteins known to contain this domain. Below are representative domain results. There are

additional areas on NOV1a that also have homology to these Domains.

Table 13E. Domain Analysis of NOV1
gnl|Pfam|pfam02535, Zip, ZIP Zinc transporter CD-Length = 152 residues,   90.8% aligned Score =   134 bits (338), Expect = 1e-32

Table 13F. Domain Analysis of NOV1
gnl|Pfam|pfam01027, UPF0005, Uncharacterized protein family UPF0005 CD-Length = 188 residues, only 63.8% aligned Score = 36.6 bits (83), Expect = 0.005

10

Estrogen responses of human breast cancer cell lines have frequently been shown to be

promoted by insulin. The action of insulin, and its interaction with estradiol, regulates the

expression of the estrogen-induced genes, LIV-1 and pS2. Both hormones cause increases in

mRNA levels of the two genes but do so by distinct mechanisms. The concentration of insulin

15   required to produce this effect suggests that it is acting via its ability to bind to the IGF-1

receptor. Both insulin and estradiol exert their effects at the level of transcription. Induction by

insulin is dependent upon continued protein synthesis whereas induction by estradiol is not.

Induction by both insulin and estradiol is prevented by the pure antiestrogen. ICI 164384,

indicating the requirement for an activatable estrogen receptor. Insulin does not stimulate LIV-

20   1 expression via the androgen receptor. These results demonstrate that both estradiol and

insulin can stimulate the transcription of these estrogen-inducible genes, by separate

mechanisms both of which involve the estrogen receptor. (See El-Tanani et al., 1997, J.

Steroid Biochem Mol Biol. 60:269).

Investigation of the protein product of the estrogen-regulated gene LIV-1, implicated

25   in metastatic breast cancer, has revealed 10 protein sequences of unknown function that

belong to a new family with potential to control intracellular Zn2+ homeostasis. Sequence

alignment highlights the similarity in transmembrane domains and extramembrane charged

residues, indicating potential ion-transport ability. This family has a novel highly conserved

motif of 66 residues, including a transmembrane domain and a catalytic zinc-binding sequence

of zinc metalloproteases, containing conserved (indicated in bold type) proline and glutamine residues. These proteins contain more plentiful histidine-rich repeats than zinc transporters, suggesting an ability to bind or transport zinc across membranes. These 11 proteins may form a new family with the potential to control intracellular Zn2+ homeostasis. (See Taylor, 2000,

5   IUBMB Life, 49:249).

The disclosed NOV1 nucleic acid of the invention encoding a Human LIV-1 -like protein includes the nucleic acid whose sequence is provided in Table 13A or a fragment thereof. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 13A while still encoding a protein

10   that maintains its Human LIV-1-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include

15   chemical modifications. Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject. In the mutant or variant nucleic

20   acids, and their complements, up to about 0 percent of the bases may be so changed.

The disclosed NOV1 protein of the invention includes the Human LIV-1-like protein whose sequence is provided in Table 13B. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in Table 13B while still encoding a protein that maintains its Human LIV-1-like activities and

25   physiological functions, or a functional fragment thereof. In the mutant or variant protein, up to about 0% percent of the residues may be so changed.

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to any of the proteins of the invention.

The above defined information for this invention suggests that this Human LIV-1-like

30   protein (NOV1) may function as a member of a "Human LIV-1 family". Therefore, the NOV1 nucleic acids and proteins identified here may be useful in potential therapeutic applications implicated in (but not limited to) various pathologies and disorders as indicated below. The potential therapeutic applications for this invention include, but are not limited to: protein therapeutic, small molecule drug target, antibody target (therapeutic, diagnostic, drug

targeting/cytotoxic antibody), diagnostic and/or prognostic marker, gene therapy (gene delivery/gene ablation), research tools, tissue regeneration *in vivo* and *in vitro* of all tissues and cell types composing (but not limited to) those defined here.

The NOV1 nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in cancer including but not limited to various pathologies and disorders as indicated below. For example, a cDNA encoding the Human LIV-1-like protein (NOV1) may be useful in gene therapy, and the Human LIV-1 -like protein (NOV1) may be useful when administered to a subject in need thereof. By way of nonlimiting example, the compositions of the present invention will have efficacy for treatment of patients suffering from ; cancer, especially breast, ovarian and bladder cancer, and/or other pathologies or conditions. The NOV1 nucleic acid encoding the Human LIV-1-like protein of the invention, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

NOV1 nucleic acids and polypeptides are further useful in the generation of antibodies that bind immuno-specifically to the novel NOV1 substances for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-NOVX Antibodies" section below. The disclosed NOV1 proteins have multiple hydrophilic regions, each of which can be used as an immunogen. These novel proteins can be used in assay systems for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders.

NOV2

A disclosed NOV2 nucleic acid of 3851 nucleotides identified herein as SEQ ID NO: 27 (also referred to as CG56149-01) encoding a novel Human NRD convertase-like protein is shown in Table 14A.

Table 14A. NOV2 nucleotide sequence (SEQ ID NO:27).

```
AGACTGGGGTGGGGGAGGGGTTCAGGCCTGTTCCCCGCGGCTGCGGCAGCACCAGGGCCGGCCGCCACCGCCTCTAGAAC
GCGGAGGAGGTGGGTCCTGGGAAGCGGGATGTCCATCGCTCCAGCTTGGTGGTGAATGCTGAGGAGAGTCACTGTTGCTG
CAGTCTGTGCCACCCGGAGGAAGTTGTGTGAGGCCGGGCGGGACGTCGCGGCGCTCTGGGGAATCGAAACGCGGGGTCGG
TGCGAAGACTCTGCTGCTGCCAGACCCTTTCCTATTCTGGCCATGCCTGGAAGGAACAAGGCGAAGTCTACCTGCAGCTG
CCCTGACCTGCAGCCCAATGGACAGGATCTGGGCGAGAACAGCCGGGTTGCCCGTCTAGGAGCGGATGAATCTGAGGAAG
AGGGACGGAGGGGGTCTCTCAGTAATGCTGGGGACCCTGAGATCGTCAAGTCTCCCAGCGACCCCAAGCAATACCGATAC
ATCAAATTACAGAATGGCCTACAGGCACTTCTGATTTCAGACCTAAGTAATATGGAAGGTAAAACAGGAAATACAACAGA
TGATGAAGAAGAAGAGGAGGTGGAGGAAGAAGAAGAAGATGATGATGAAGATTCTGGAGCTGAAATAGAAGATGACGATG
AAGAGGGTTTTGATGATGAAGATGAGTTTGATGATGAACATGATGATGATCTTGATACTGAGGATAATGAATTGGAAGAA
TTAGAAGAGAGAGCAGAAGCTAGAAAAAAAACTACTGAAAAACAGCAATTGCAGAGCCTGTTTTTGCTGTGGTCAAAGCT
GACTGATAGACTGTGGTTTAAGTCAACTTATTCAAAAATGTCTTCAACCCTGCTGGTCGAGACAAGAAATCTTTATGGGG
TAGTTGGAGCTGAAAGCAGGTCTGCACCTGTTCAGCATTTGGCAGGATGGCAAGCGGAGGAGCAGCAGGGTGAAACTGAC
ACAGTTCTGTCTGCAGCGGCTCTTTGTGTTGGAGTTGGGAGTTTCGCTGATCCAGATGACCTGCCGGGGCTGGCACACTT
TTTGGAGCACATGGTATTCATGGGTAGTTTGAAATATCCAGATGAGAATGGATTTGATGCCTTCCTGAAGAAGCATGGGG
GTAGTGATAATGCCTCAACTGATTGTGAACGCACTGTCTTTCAGTTTGATGTCCAGAGGAAGTACTTCAAGGAAGCTCTT
GATAGATGGGCGCAGTTCTTCATCCACCCACTAATGATCAGAGATGCAATTGACCGTGAAGTTGAAGCTGTTGATAGTCA
ATATCAACTTGCAAGGCCTTCTGATGCAAACAGAAAGGAAATGTTGTTTGGAAGCCTTGCTAGACCTGGCCATCCTATGG
GAAAATTTTTTTGGGGAAATGCTGAGACGCTCAAGCATGAGCCAAGAAAGAATAATATTGATACACATGCTAGATTGAGA
GAATTCTGGATGCGTTACTACTCTTCTCATTACATGACTTTAGTGGTTCAATCCAAAGAAACACTGGATACTTTGGAAAA
GTGGGTGACTGAAATCTTCTCTCAGATACCAAACAATGGGTTACCCAGACCAAACTTTGGCCATTTAACGGATCCATTTG
ACACACCAGCATTTAACAAACTTTATAGAGTTGTTCCAATCAGAAAAATTCATGCTCTGACCATCACATGGGCACTTCCT
CCTCAACAGCAACATTACAGGGTGAAGCCACTTCATTATATATCCTGGCTGGTTGGACATGAAGGCAAAGGCAGCATTCT
TTCTTTCCTTAGGAAAAAATGCTGGGCTCTTGCACTGTTTGGTGGAAATGGTGAGACAGGATTTGAGCAAAATTCTACTT
ATTCAGTGTTCAGCATTTCTATTACATTGACTGATGAGGGTTATGAACATTTTTATGAGGTTGCTTACACTGTCTTTCTG
TATTTAAAAATGCTGCAGAAGCTAGGCCCAGAAAAAAGAATTTTTGAAGAGATTCGGAAAATTGAGGATAATGAATTTCA
TTACCAAGAACAGACAGATCCAGTTGAGTATGTGGAAAACATGTGTGAGAACATGCAGCTGTACCCATTGCAGGACATTC
TCACTGGAGATCAGCTTCTTTTTGAATACAAGCCAGAAGTCATTGGTGAAGCCTTGAATCAGCTAGTTCCTCAAAAAGCA
AATCTTGTTTTACTGTCTGGTGCTAATGAGGGAAAATGTGACCTCAAGGAGAAATGGTTTGGAACTCAATATAGTATAGA
AGATATTGAAAACTCTTGGGCTGAACTGTGGAATAGTAATTTCGAATTAAATCCAGATCTTCATCTTCCAGCTGAAAACA
AGTACATAGCCACGGACTTTACGTTGAAGGCTTTCGATTGCCCGGAAACAGAATACCCAGTTAAAATTGTGAATACTCCA
CAAGGTTGCCTGTGGTATAAGAAAGACAACAAATTCAAAATCCCCAAAGCATATATACGTTTCCATCTAATTTCACCGTT
GATACAGAAATCTGCAGCAAATGTGGTCCTCTTTGATATCTTTGTCAATATCCTTACGCATAACCTTGCGGAACCAGCTT
ATGAAGCAGATGTGGCACAGCTGGAGTATAAACTGGCAGCTGGAGAACATGGTTTAATTATTCGAGTGAAAGGATTTAAC
CACAAACTACCTCTACTGTTTCAGCTCATTATTGACTACTTAGCTGAGTTCAATTCCACACCAGCTGTCTTTACAATGAT
AACTGAGCAGTTGAAGAAGACCTACTTTAACATCCTCATCAAGCCTGAGACTTTGGCCAAAGATGTACGGCTTTTAATCT
TGGAATATGCCCGTTGGTCTATGATTGACAAGTACCAGGCTTTGATGGACGGCCTTTCCCTTGAGTCTCTGCTGAGCTTC
GTCAAAGAATTCAAATCCCAGCTCTTTGTGGAGGGCCTGGTACAAGGGAATGTCACAAGCACGAATCTATGGATTTCCT
GAAATATGTTGTTGACAAACTAAACTTCAAGCCTCTGGAGCAGGAGATGCCTGTGCAGTTCCAGGTGGTAGAGCTGCCCA
GTGGCCACCATCTATGCAAAGTGAAAGCTCTGAACAAGGGTGATGCCAACTCTGAAGTCACTGTGTACTACCAGTCAGGT
ACCAGGAGTCTAAGAGAATATACGCTTATGGAGCTGCTTGTGATGCACATGGAAGAACCTTGTTTTGACTTCCTTCGAAC
CAAGCAGACCCTTGGGTACCATGTCTACCCTACCTGTAGGAACACATCCGGGATTCTAGGATTTTCTGTCACTGTGGGGA
CTCAGGCAACCAAATACAATTCTGAAGTTGTTGATAAGAAGATAGAAGAGTTTCTTTCTAGCTTTGAGGAGAAGATTGAG
AACCTCACTGAAGAGGCATTCAACACCCAGGTCACAGCTCTCATCAAGCTGAAGGAGTGTGAGGATACCCACCTTGGGGA
GGAGGTGGATAGGAACTGGAATGAAGTGGTTACACAGCAGTACCTCTTTGACCGCCTTGCCCACGAGATTGAAGCACTGA
AGTCATTCTCAAAATCAGACCTGGTCAACTGGTTCAAGGCTCATAGAGGGGCCAGGAAGTAAAATGCTCAGCGTTCATGTT
GTTGGGTATGGGAAGTATGAACTGGAAGAGGATGGATCCCCTTCTAGTGAGGATTCAAATTCTTCTTGTGAAGTGATGCA
GCTGACCTACCTGCCAACCTCTCCTCTGCTGGCAGATTGTATCATCCCCATTACTGATATCAGGGCTTTCACAACAACAC
TCAACCTTCTCCCCTACCATAAAATAGTCAAATAAATAAACTGCAGTCACGTTGGCCTGAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAA
```

A NOV2 polypeptide (SEQ ID NO:28) encoded by SEQ ID NO:27 has 1219 amino acid residues and is presented using the one-letter code in Table 14B. Signal P, Psort and/or Hydropathy results predict that NOV2 contains no signal peptide and is likely to be localized to the endoplasmic reticulum (membrane) with a certainty of 0.5500. In other embodiments, NOV2 may also be localized to the lysosome (lumen) with a certainty of 0.1900, the microbody with a certainty of 0.1868, or the endoplasmic reticulum (lumen) with a certainty of 0.1000. The most likely cleavage site for NOV2 is between positions 18 and 19: KLC-EA.

Table 14B. Encoded NOV2 protein sequence (SEQ ID NO:28).

```
MLRRVTVAAVCATRRKLCEAGRDVAALWGIETRGRCEDSAAARPFPILAMPGRNKAKSTCSCPDLQPNGQDLGENSRVAR
```

```
LGADESEEEGRRGSLSNAGDPEIVKSPSDPKQYRYIKLQNGLQALLISDLSNMEGKTGNTTDDEEEEEVEEEEEDDDEDS
GAEIEDDDEEGFDDEDEFDDEHDDDLDTEDNELEELEERAEARKKTTEKQQLQSLFLLWSKLTDRLWFKSTYSKMSSTLL
VETRNLYGVVGAESRSAPVQHLAGWQAEEQQGETDTVLSAAALCVGVGSFADPDDLPGLAHFLEHMVFMGSLKYPDENGF
DAFLKKHGGSDNASTDCERTVFQFDVQRKYFKEALDRWAQFFIHPLMIRDAIDREVEAVDSEYQLARPSDANRKEMLFGS
LARPGHPMGKFFWGNAETLKHEPRKNNIDTHARLREFWMRYYSSHYMTLVVQSKETLDTLEKWVTEIFSQIPNNGLPRPN
FGHLTDPFDTPAFNKLYRVVPIRKIHALTITWALPPQQQHYRVKPLHYISWLVGHEGKGSILSFLRKKCWALALFGGNGE
TGFEQNSTYSVFSISITLTDEGYEHFYEVAYTVFLYLKMLQKLGPEKRIFEEIRKIEDNEFHYQEQTDPVEYVENMCENM
QLYPLQDILTGDQLLFEYKPEVIGEALNQLVPQKANLVLLSGANEGKCDLKEKWFGTQYSIEDIENSWAELWNSNFELNP
DLHLPAENKYIATDFTLKAFDCPETEYPVKIVNTPQGCLWYKKDNKFKIPKAYIRFHLISPLIQKSAANVVLFDIFVNIL
THNLAEPAYEADVAQLEYKLAAGEHGLIIRVKGFNHKLPLLFQLIIDYLAEFNSTPAVFTMITEQLKKTYFNILIKPETL
AKDVRLLILEYARWSMIDKYQALMDGLSLESLLSFVKEFKSQLFVEGLVQGNVTSTESMDFLKYVVDKLNFKPLEQEMPV
QFQVVELPSGHHLCKVKALNKGDANSEVTVYYQSGTRSLREYTLMELLVMHMEEPCFDFLRTKQTLGYHVYPTCRNTSGI
LGFSVTVGTQATKYNSEVVDKKIEEFLSSFEEKIENLTEEAFNTQVTALIKLKECEDTHLGEEVDRNWNEVVTQQYLFDR
LAHEIEALKSFSKSDLVNWFKAHRGPGSKMLSVHVVGYGKYELEEDGSPSSEDSNSSCEVMQLTYLPTSPLLADCIIPIT
DIRAFTTTLNLLPYHKIVK
```

NOV2 is localized on chromosome 1p32.2-34.4 and is expressed in at least the following tissues: multiple cancers and skeletal muscle. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, and/or RACE sources.

NOV2 has homology to the amino acid sequences shown in the BLASTP data listed in Table 14C.

Table14C. BLAST results for NOV2					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
ref|NP_002516.1| (NM_002525)	nardilysin (N-arginine dibasic convertase) 1 [Homo sapiens]	1219	100	100	0.0
emb|CAA63696.1| (X93208)	NRD2 convertase [Rattus sp.]	1229	90	90	0.0
ref|XP_001517.5| (XM_001517)	nardilysin (N-arginine dibasic convertase) [Homo sapiens]	1017	99	99	0.0
emb|CAB72328.1| (AL050343)	dJ657D16.1 (nardilysin (N-arginine dibasic convertase)) [Homo sapiens]	862	99	99	0.0
gb|AAF48105.1| (AE003487)	CG2025 gene product [Drosophila melanogaster]	1077	36	57	e-165

The homology of these sequences is shown graphically in the ClustalW analysis shown in Table 14D.

Table 14D. ClustalW Analysis of NOV2

```
1)  NOV2 (SEQ ID NO:28)
2)  ref|NP_002516.1|  (SEQ ID NO:106)
3)  emb|CAA63696.1|   (SEQ ID NO:107)
4)  ref|XP_001517.5|  (SEQ ID NO:108)
5)  emb|CAB72328.1|   (SEQ ID NO:109)
6)  gb|AAF48105.1|    (SEQ ID NO:110)
```

```
                      10        20        30        40        50
                 ....|....|....|....|....|....|....|....|....|....|
NOV2             MLRRVTVAAVCATRRKL-CEAGRDVAALWGIETRGRCEDSAAARPFPILA
ref|NP_002516.1| MLRRVTVAAVCATRRKL-CEAGRDVAALWGIETRGRCEDSAAARPFPILA
emb|CAA63696.1|  MLRRVAVAAVFATGRKLRCEAGRDVTAVGRIEARGLCEES--AKPFPTLT
ref|XP_001517.5| MLRRVTVAAVCATRRKL-CEAGRELAALWGIETRGRCEDSAAARPFPILA
emb|CAB72328.1|  -------------------------------------------------
gb|AAF48105.1|   -------------------------------------------------

                      60        70        80        90        100
                 ....|....|....|....|....|....|....|....|....|....|
NOV2             MPGRNKAKSTCSCPDLQPNGQDLGENSRVARLGADESEEEGRRGSLSNAG
ref|NP_002516.1| MPGRNKAKSTCSCPDLQPNGQDLGENSRVARLGADESEEEGRRGSLSNAG
emb|CAA63696.1|  MPGRNKAKSTCSCPDLQPNGQDLGESGRVARLGADESEEEGR--SLSNVG
ref|XP_001517.5| MPGRNKAKSTCSCPDLQPNGQDLGENSRVARLGADESEEEGRRGSLSNAG
emb|CAB72328.1|  -------------------------------------------------
gb|AAF48105.1|   ----------------------MTDQVKYLDIPE---------------

                      110       120       130       140       150
                 ....|....|....|....|....|....|....|....|....|....|
NOV2             DPEIVKSPSDPKQYRYIKLQNGLQALLISDLSNMEGKTGNTTDEEEEEEV
ref|NP_002516.1| DPEIVKSPSDPKQYRYIKLQNGLQALLISDLSNMEGKTGNTTDEEEEEEV
emb|CAA63696.1|  DPEIIKSPSDPKQYRYIKLQNGLQALLISDLSNVEGKTGNATDEEEEEEE
ref|XP_001517.5| DPEIVKSPSDPKQYRYIKLQNGLQALLISDLSNMEGKTGNTTDEEEEEEV
emb|CAB72328.1|  -------------------------------------------------
gb|AAF48105.1|   -----KSEIDKKLYKTELLGNGLHALIVSDPSPMP--------------

                      160       170       180       190       200
                 ....|....|....|....|....|....|....|....|....|....|
NOV2             EEEE-----------EDDDEDSGAEIEDDDEEGFDDEDEFDDEH--DDD
ref|NP_002516.1| EEEE-----------EDDDEDSGAEIEDDDEEGFDDEDEFDDEH--DDD
emb|CAA63696.1|  EEEEGEEEEEEEEDDDEDDDEDSGAEIQDDDEEGFDDEEEFDDDEHDDDD
ref|XP_001517.5| EEEE-----------EDDDEDSGAEIEDDDEEGFDDEDEFDDEH--DDD
emb|CAB72328.1|  -------------------------------------------------
gb|AAF48105.1|   -------------------------HDGFTTSESSSSKS----------

                      210       220       230       240       250
                 ....|....|....|....|....|....|....|....|....|....|
NOV2             LDTEDNELEELEERAEARKKTTEKQQLQSLFLLWSKLTDRLWFKSTYSKM
ref|NP_002516.1| LDTEDNELEELEERAEARKKTTEKQQLQSLFLLWSKLTDRLWFKSTYSKM
emb|CAA63696.1|  LDNEDNELEELEERVEARKKTTEKQQSQNLFLLWSKLTDRLWFKSSYSKM
ref|XP_001517.5| LDTEDNELEELEERAEARKKTTEKQQLQSLFLLWSKLTDRLWFKSTYSKM
emb|CAB72328.1|  -------------------------------------------------
gb|AAF48105.1|   -----------------------------------TVS------------

                      260       270       280       290       300
                 ....|....|....|....|....|....|....|....|....|....|
NOV2             SSTLLVETRNLYGVVGAESRSAPVQHLAGWQAEEQQGETDTVLSAAALCV
ref|NP_002516.1| SSTLLVETRNLYGVVGAESRSAPVQHLAGWQAEEQQGETDTVLSAAALCV
emb|CAA63696.1|  SSTLLVETRNLYGVVGAESRSAPVEHLAGWQVEEQQGETDTVLSAAALCV
ref|XP_001517.5| SSTLLVETRNLYGVVGAESRSAPVQHLAGWQAEEQQGETDTVLSAAALCV
emb|CAB72328.1|  -------------------------------------------------
gb|AAF48105.1|   TSSSIESRSESTSSTSTDSESS--------E-ESSSEEGDEKLAACALLE

                      310       320       330       340       350
                 ....|....|....|....|....|....|....|....|....|....|
NOV2             GVGSFADPDDLPGLAHFLEHMVFMGSLKYPDENGFDAFLKKHGGSDNAST
ref|NP_002516.1| GVGSFADPDDLPGLAHFLEHMVFMGSLKYPDENGFDAFLKKHGGSDNAST
emb|CAA63696.1|  GVGSFADPDDLPGLAHFLEHMVFMGSLKYPDENGFDAFLKKHGGSDNAST
ref|XP_001517.5| GVGSFADPDDLPGLAHFLEHMVFMGSLKYPDENGFDAFLKKHGGSDNAST
emb|CAB72328.1|  -------------------------------------------------
gb|AAF48105.1|   DYGSFAEPTKYQGLAHFLEHMIFMGSEKYPKENIFDAHIKKCGGFANANT

                      360       370       380       390       400
                 ....|....|....|....|....|....|....|....|....|....|
NOV2             DCERTVFQFDVQRKYFKEALDRWAQFFIHPLMIRDAIDREVEAVDSEYQL
ref|NP_002516.1| DCERTVFQFDVQRKYFKEALDRWAQFFIHPLMIRDAIDREVEAVDSEYQL
```

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emb|CAA63696.1|   DCERTVFQFDVQRKYFKEALDRWAQFFIHPLMIRDAIDREVEAVDSEYQL
ref|XP_001517.5|  DCERTVFQFDVQRKYFKEALDRWAQFFIHPLMIRDAIDREVEAVDSEYQL
emb|CAB72328.1|   --------------------WAQFFIHPLMIRDAIDREVEAVDSEYQL
gb|AAF48105.1|    DCEDTVFYFEVAEKHLDSSLDYETALMKAPLMKQEAMQRERSAVDSEFQQ

                       410       420       430       440       450
                  ....|....|....|....|....|....|....|....|....|....|
NOV2              ARPSDANRKEMLFGSLARPGHPMGKFFWGNAETLKHEPRKNNIDTHARLR
ref|NP_002516.1|  ARPSDANRKEMLFGSLARPGHPMGKFFWGNAETLKHEPRKNNIDTHARLR
emb|CAA63696.1|   ARPSDANRKEMLFGSLARPGHPMGKFFWGNAETLKHEPRKNNIDTHARLR
ref|XP_001517.5|  ARPSDANRKEMLFGSLARPGHPMGKFFWGNAETLKHEPRKNNIDTHARLR
emb|CAB72328.1|   ARPSDANRKEMLFGSLARPGHPMGKFFWGNAETLKHEPRKNNIDTHARLR
gb|AAF48105.1|    ILQDDETRRDQLLASLATKGFPHCTFAWGNMKSLKEN--VDDAELHKILH

                       460       470       480       490       500
                  ....|....|....|....|....|....|....|....|....|....|
NOV2              EFWMRYYSSHYMTLVVQSKETLDTLEKWVTEIFSQIPNNGLPRPNFG--H
ref|NP_002516.1|  EFWMRYYSSHYMTLVVQSKETLDTLEKWVTEIFSQIPNNGLPRPNFG--H
emb|CAA63696.1|   EFWMRYYSSHYMTLVVQSKETLDTLEKWVTEIFSQIPNNGLPRPNFS--H
ref|XP_001517.5|  EFWMRYYSSHYMTLVVQSKETLDTLEKWVTEIFSQIPNNGLPRPNFG--H
emb|CAB72328.1|   EFWMRYYSSHYMTLVVQSKETLDTLEKWVTEIFSQIPNNGLPRPNFG--H
gb|AAF48105.1|    EIRKEHYGANRMYVCIQARLPIDELESLVVRHFSGIPHNEVKAPDLSSFN

                       510       520       530       540       550
                  ....|....|....|....|....|....|....|....|....|....|
NOV2              LTDPFDTPAFNKLYRVVPIRKIHALTITWALPPQQQHYRVKPLHYISWLV
ref|NP_002516.1|  LTDPFDTPAFNKLYRVVPIRKIHALTITWALPPQQQHYRVKPLHYISWLV
emb|CAA63696.1|   LTDPFDTPAFNKLYRVVPIRKIHALTITWALPPQQQHYRVKPLHYISWLV
ref|XP_001517.5|  LTDPFDTPAFNKLYRVVPIRKIHALTITWALPPQQQHYRVKPLHYISWLV
emb|CAB72328.1|   LTDPFDTPAFNKLYRVVPIRKIHALTITWALPPQQQHYRVKPLHYISWLV
gb|AAF48105.1|    YKDAFKAEFHEQVFFVKPVENETKLEITWVLPNVRQYYRSKPDQETSYLL

                       560       570       580       590       600
                  ....|....|....|....|....|....|....|....|....|....|
NOV2              GHEGKGSILSFLRKKCWALALFGGNGETGFEQNSTYSVFSISITLTDEGY
ref|NP_002516.1|  GHEGKGSILSFLRKKCWALALFGGNGETGFEQNSTYSVFSISITLTDEGY
emb|CAA63696.1|   GHEGKGSILSYLRKKCWALALFGGNGETGFEQNSTYSVFSISITLTDEGY
ref|XP_001517.5|  GHEGKGSILSFLRKKCWALALFGGNGETGFEQNSTYSVFSISITLTDEGY
emb|CAB72328.1|   GHEGKGSILSFLRKKCWALALFGGNGETGFEQNSTYSVFSISITLTDEGY
gb|AAF48105.1|    GYEGRGSLCAVLRRRLWALQLIAGIDENGFDMNSMYSLFNICIYLTDEGE

                       610       620       630       640       650
                  ....|....|....|....|....|....|....|....|....|....|
NOV2              EHFYEVAYTVFLYLKMLQKLGPEKRIFEEIRKIEDNEFHYQEQTDPVEYV
ref|NP_002516.1|  EHFYEVAYTVFLYLKMLQKLGPEKRIFEEIRKIEDNEFHYQEQTDPVEYV
emb|CAA63696.1|   EHFYEVAHTVFQYLKMLQKLGPEKRVFEEIQKIEDNEFHYQEQTDPVEYV
ref|XP_001517.5|  EHFYEVAYTVFQYLKMLQKLGPEKRIFEEIRKIEDNEFHYQEQTDPVEYV
emb|CAB72328.1|   EHFYEVAYTVFQYLKMLQKLGPEKRIFEEIRKIEDNEFHYQEQTDPVEYV
gb|AAF48105.1|    KNLDEVLAATFAYVKLFANCGSMKDVYEEQQRNEETGFREHAQRPAFDNV

                       660       670       680       690       700
                  ....|....|....|....|....|....|....|....|....|....|
NOV2              ENMCENMQLYPLQDILTGDQLLFEYKPEVIGEALNQLVPQKANLVLLSG-
ref|NP_002516.1|  ENMCENMQLYPLQDILTGDQLLFEYKPEVIGEALNQLVPQKANLVLLSG-
emb|CAA63696.1|   ENMCENMQLYPRQDFLTGDQLLFEYKPEVIAEALNQLVPQKANLVLLSG-
ref|XP_001517.5|  ENMCENMQLYPLQDILTGDQLLFEYKPEVIGEALNQLVPQKANLVLLSG-
emb|CAB72328.1|   ENMCENMQLYPLQDILTGDQLLFEYKPEVIGEALNQLVPQKANLVLLSG-
gb|AAF48105.1|    QELVLNLKYEPPKDILTGKELYMEYNEEHLKELTSHLNEMKFNLMVTSRR

                       710       720       730       740       750
                  ....|....|....|....|....|....|....|....|....|....|
NOV2              --ANEGKCDLKEKWFGTQYSIEDIENSWAELWNSNFELNPDLHLPAENKY
ref|NP_002516.1|  --ANEGKCDLKEKWFGTQYSIEDIENSWAELWNSNFELNPDLHLPAENKY
emb|CAA63696.1|   --ANEGRCDLKEKWFGTQYSIEDIENSWTELWKSNFDLNSDLHLPAENKY
ref|XP_001517.5|  --ANEGKCDLKEKWFGTQYSIEDIENSWAELWNSNFELNPDLHLPAENKY
emb|CAB72328.1|   --ANEGKCDLKEKWFGTQYSIEDIENSWAELWNSNFELNPDLHLPAENKY
gb|AAF48105.1|    KYDDISAYDKTEEWFGTEYATIPMPEKWRKLWEDSVPL-PELFLPESNKY

                       760       770       780       790       800
                  ....|....|....|....|....|....|....|....|....|....|
NOV2              IATDFTLKAFDCPETEYP---VKIVNTPQGCLWYKKDNKFKIPKAYIRFH
ref|NP_002516.1|  IATDFTLKAFDCPETEYP---VKIVNTPQGCLWYKKDNKFKIPKAYIRFH
emb|CAA63696.1|   IATDFTLKAFDCPETEYP---AKIVNTPQGCLWYKKDNKFKIPKAYIRFH
ref|XP_001517.5|  IATDFTLKAFDCPETEYP---VKIVNTPQGCLWYKKDNKFKIPKAYIRFH
emb|CAB72328.1|   IATDFTLKAFDCPETEYP---VKIVNTPQGCLWYKKDNKFKIPKAYIRFH
gb|AAF48105.1|    VTDDFTLHWHSMGRPEVPDSPKLLIKTDTCELWFRQDDKFDTPEAHMAFY

                       810       820       830       840       850
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91

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                    ....|....|....|....|....|....|....|....|....|....|
NOV2                LISPLIQKSAANVVLFDIFVNILTHNLAEPAYEADVAQLEYKLAAGEHGL
ref|NP_002516.1|    LISPLIQKSAANVVLFDIFVNILTHNLAEPAYEADVAQLEYKLAAGEHGL
emb|CAA63696.1|     LISPLIQKSAANVVLFDIFVNILTHNLAEPAYEADVAQLEYKLVAGEHGL
ref|XP_001517.5|    LISPLIQKSAANVVLFDIFVNILTHNLAEPAYEADVAQLEYKLVAGEHGL
emb|CAB72328.1|     LISPLIQKSAANVVLFDIFVNILTHNLAEPAYEADVAQLEYKLVAGEHGL
gb|AAF48105.1|      FISPMQRONAKNDAMCSLXEEMXRFHXCEELYPAISAGLSYSLSTIEKGL

                              860       870       880       890       900
                    ....|....|....|....|....|....|....|....|....|....|
NOV2                IIRVKGFNHKLPLLFQLIIDYLAEFNSTP--AVFTMITEQLKKTYFNILI
ref|NP_002516.1|    IIRVKGFNHKLPLLFQLIIDYLAEFNSTP--AVFTMITEQLKKTYFNILI
emb|CAA63696.1|     IIRVKGFNHKLPLLFQLIIDYLTEFSSTP--AVFTMITEQLKKTYFNILI
ref|XP_001517.5|    IIRVKGFNHKLPLLFQLIIDYLAEFNSTP--AVFTMITEQLKKTYFNILI
emb|CAB72328.1|     IIRVKGFNHKLPLLFQLIIDYLAEFNSTP--AVFTMITEQLKKTYFNILI
gb|AAF48105.1|      XXKVCGXNEKLHLIVEAIAEGMLNVAETLDENMLSAFVKNQRKAEFNALI

                              910       920       930       940       950
                    ....|....|....|....|....|....|....|....|....|....|
NOV2                KPETLAKDVRLLILEYARWSMIDKYQALMDGLSLESLLSFVKEFKSQLFV
ref|NP_002516.1|    KPETLAKDVRLLILEYARWSMIDKYQALMDGLSLESLLSFVKEFKSQLFV
emb|CAA63696.1|     KPETLAKDVRLLILEYSRWSMIDKYRALMDGLSLESLLNFVKDFKSQLFV
ref|XP_001517.5|    KPETLAKDVRLLILEYARWSMIDKYQALMDGLSLESLLSFVKEFKSQLFV
emb|CAB72328.1|     KPETLAKDVRLLILEYARWSMIDKYQALMDGLSLESLLSFVKEFKSQLFV
gb|AAF48105.1|      KPKALNRDIRLCVLERIRWLMINKYKCLSS-VILEDMREBAHQFPKELYX

                              960       970       980       990       1000
                    ....|....|....|....|....|....|....|....|....|....|
NOV2                EGLVQGNVTSTESMDFLKYVVDKLNFKPL-EQEMPVQFQVVELPSGHHLC
ref|NP_002516.1|    EGLVQGNVTSTESMDFLKYVVDKLNFKPL-EQEMPVQFQVVELPSGHHLC
emb|CAA63696.1|     EGLVQGNVTSTESMDFLRYVVDKLNFVPL-EREMPVQFQVVELPSGHHLC
ref|XP_001517.5|    EGLVQGNVTSTESMDFLKYVVDKLNFKPL-EQEMPVQFQVVELPSGHHLC
emb|CAB72328.1|     EGLVQGNVTSTESMDFLKYVVDKLNFKPL-EQEMPVQFQVVELPSGHHLC
gb|AAF48105.1|      QSLXQGNYXEESAHNVMNSXXSRLNCKQXRERXGRFXEDITVKLBVGTSXI

                              1010      1020      1030      1040      1050
                    ....|....|....|....|....|....|....|....|....|....|
NOV2                KVKALNKGDANSEVTVYYQSGTRSLREYTLMELLVMHMEEPCFDFLRTKQ
ref|NP_002516.1|    KVKALNKGDANSEVTVYYQSGTRSLREYTLMELLVMHMEEPCFDFLRTKQ
emb|CAA63696.1|     KVRALNKGDANSEVTVYYQSGTRSLREYTLMELLVMHMEEPCFDFLRTKQ
ref|XP_001517.5|    KVKALNKGDANSEVTVYYQ------------------------VRYQE
emb|CAB72328.1|     KVKALNKGDANSEVTVYYQSGTRSLREYTLMELLVMHMEEPCFDFLRTKQ
gb|AAF48105.1|      XCHALNVQDTNXVXINEYQIGPNXXRVESXLXLLXMFVXXEPLXDQLRTKE

                              1060      1070      1080      1090      1100
                    ....|....|....|....|....|....|....|....|....|....|
NOV2                TLGYHVYPTCRNTSGILGFSVTVGTQATKYNSEVVDKKIEEFLSSFEEKI
ref|NP_002516.1|    TLGYHVYPTCRNTSGILGFSVTVGTQATKYNSEVVDKKIEEFLSSFEEKI
emb|CAA63696.1|     TLGYHVYPTCRNTSGILGFSVTVGTQATKYNSETVDKKIEEFLSSFEEKI
ref|XP_001517.5|    SKRIXAXGAACDAHGRTLF-------------------------------
emb|CAB72328.1|     TLGYHVYPTCRNTSGILGFSVTVGTQATKYNSEVVDKKIEEFLSSFEEKI
gb|AAF48105.1|      QLGYHVGATVRLNYGIAGXSXMVNSQETKTTAXYVXGRIEVXRAKMLQIX

                              1110      1120      1130      1140      1150
                    ....|....|....|....|....|....|....|....|....|....|
NOV2                ENLTEBAFNTQVTALIKLKECEDTHLGEEVDRNWNEVVTQQYLFDRLAHE
ref|NP_002516.1|    ENLTEBAFNTQVTALIKLKECEDTHLGEEVDRNWNEVVTQQYLFDRLAHE
emb|CAA63696.1|     ENLTEBAFNTQVTALIKLKECEDTHLGEEVDRNWNEVVTQQYLFDRLAHE
ref|XP_001517.5|    --------------------------------------------------
emb|CAB72328.1|     ENLTEBAFNTQVTALIKLKECEDTHLGEEVDRNWNEVVTQQYLFDRLAHE
gb|AAF48105.1|      RHXPQDEXEHTRDSLIKLKLVADLALSTEXSRNWDEXXNXSYLFDRRRRQ

                              1160      1170      1180      1190      1200
                    ....|....|....|....|....|....|....|....|....|....|
NOV2                IEALKSFSK---SDLVNWFKAHRGPGSKMLSVHVVGYGKYELEEDGSPSS
ref|NP_002516.1|    IEALKSFSK---SDLVNWFKAHRGPGSKMLSVHVVGYGKYELEEDGSPSS
emb|CAA63696.1|     IEALKSFSK---SDLVSWFKAHRGPGSKMLSVHVVGYGKYELEEDGAPVC
ref|XP_001517.5|    --------------------------------------------------
emb|CAB72328.1|     IEALKSFSK---SDLVNWFKAHRGPGSKMLSVHVVGYGKYELEEDGTPSS
gb|AAF48105.1|      IEVXGHRPAGMPEPXCGEDTAKCASKSDDESESENDDDDDEDEEEEESSE

                              1210      1220      1230      1240      1250
                    ....|....|....|....|....|....|....|....|....|....|
NOV2                EDSNSS--------------CEXMQLTYLPXSPLLADCIIPITDIRAFT
ref|NP_002516.1|    EDSNSS--------------CEXMQLTYLPTSPLLADCIIPITDIRAFT
emb|CAA63696.1|     EDPNS---------------REXMQLIYLPPSPLLAESTTPITDIRAFT
ref|XP_001517.5|    -------------------------------------------------
emb|CAB72328.1|     EDSNSS--------------CEXMQLTYLPTSPLLADCIIPITDIRAFT
```

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gb|AAF48105.1|        EEEEEEKEKEGLKGEDEDDLFYSENKLNVFLAKFNNAFILTDIEKK

                               1260      1270      1280
                      ....|....|....|....:|....|....|.
NOV2                  TTLNLLPYHKIVK-----------------
ref|NP_002516.1|      TTLNLLPYHKIVK-----------------
emb|CAA63696.1|       ATLSLFPYHKIVK-----------------
ref|XP_001517.5|      -----------------------------
emb|CAB72328.1|       TTLNLLPYHKIVK-----------------
gb|AAF48105.1|        DDQYGYPQQKTQPKEEDELISAHIADAIRQV
```

Table 14E lists the domain description from DOMAIN analysis results against NOV2.
This indicates that the NOV2 sequence has properties similar to those of other proteins known
to contain this domain.

Table 14E. Domain Analysis of NOV2
gnl|Pfam|pfam00675, Peptidase_M16, Insulinase (Peptidase family M16). CD-Length = 149 residues, 91.9% aligned Score = 139 bits (349), Expect = 1e-33

N-arginine dibasic convertase (NRD convertase) (accession number L27124) is a
metalloendopeptidase from rat brain cortex and testis which cleaves peptide substrates on the
N-terminus of arginine residues in basic doublets. Its predicted amino acid sequence contains a
putative zinc binding motif in a region which exhibits 35% and 48% similarity with E coli
protease III (pitrilysin E.C 3.4.99.44) and rat or human insulinase (E.C 3.4.99.45) respectively.
This feature clearly classifies this endopeptidase as a member of the pitrilysin family of zinc-
metalloproteases. However, the NRD convertase sequence contains a distinctive additional
feature consisting of a 71 acidic amino acid stretch. Its substrate selectivity and the
characteristic motifs of its amino acid sequence allow us to propose this new
metalloendopeptidase as the first member of a new class of processing enzymes. (See
Chesneau et al., 1994, Biochimie 76:234).

Heparin-binding epidermal growth factor-like growth factor (HB-EGF), a mitogen and
chemotactic factor, binds to two receptor tyrosine kinases, erbB1 and erbB4. Now we
demonstrate that HB-EGF also binds to a novel 140 kDa receptor on MDA-MB 453 cells.
Purification of this receptor showed it to be identical to N-arginine dibasic convertase (NRDc),
a metalloendopeptidase of the M16 family. Binding to cell surface NRDc and NRDc in
solution was highly specific for HB-EGF among EGF family members. When overexpressed
in cells, NRDc enhanced their migration in response to HB-EGF but not to EGF. Conversely,
inhibition of endogenous NRDc expression in cells by antisense morpholino oligonucleotides
inhibited HB-EGF-induced cell migration. Anti-erbB1 neutralizing antibodies completely
abrogated the ability of NRDc to enhance HB-EGF-dependent migration, demonstrating that
this NRDc activity was dependent on erbB1 signaling. Although NRDc is a metalloproteinase,

enzymatic activity was not required for HB-EGF binding or enhancement of cell migration; neither did NRDc cleave HB-EGF. Together, these results suggest that NRDc is a novel specific receptor for HB-EGF that modulates HB-EGF-induced cell migration via erbB1. (See Nishi et al., 2001, EMBO J 20:3342).

5   The disclosed NOV2 nucleic acid of the invention encoding a Human NRD convertase-like protein includes the nucleic acid whose sequence is provided in Table 14A or a fragment thereof. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 14A while still encoding a protein that maintains its Human NRD convertase-like activities and physiological functions,

10   or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, by way of nonlimiting example,

15   modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject. In the mutant or variant nucleic acids, and their complements, up to about 0 percent of the bases may be so changed.

20   The disclosed NOV2 protein of the invention includes the Human NRD convertase - like protein whose sequence is provided in Table 14B. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in Table 14B while still encoding a protein that maintains its Human NRD convertase-like activities and physiological functions, or a functional fragment thereof. In the mutant or

25   variant protein, up to about 0 percent of the residues may be so changed.

The NOV2 nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in diabetes, metabolic disorders and/or other pathologies and disorders.

NOV2 nucleic acids and polypeptides are further useful in the generation of antibodies

30   that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-NOVX Antibodies" section below. The disclosed NOV2 protein has multiple hydrophilic regions, each of which can be used as an immunogen. These novel proteins can be used in assay systems for

functional analysis of various human disorders, which are useful in understanding of

pathology of the disease and development of new drug targets for various disorders.

NOV3

5   A disclosed NOV3 nucleic acid of 2038 nucleotides identified herein as SEQ ID

NO:29 (also referred to as CG55155-01) encoding a novel Kallikrein-like protein is shown in

Table 15A.

Table 15A. NOV3 Nucleotide Sequence (SEQ ID NO: 29)
GTTTTCAGAATGATTTTATTCAAGCAAGCAACTTATTTCATTTCCTTGTTTGCTACAGTTTCCTGTGGATGTCTGACTCA ACTCTATGAAAACGCCTTCTTCAGAGGTGGGGATGTAGCTTCCATGTACACCCCAAATGCCCAATACTGCCAGATGAGGT GCACATTCCACCCAAGGTGTTTGCTATTCAGTTTTCTTCCAGCAAGTTCAATCAATGACATGGAGAAAAGGTTTGGTTGC TTCTTGAAAGATAGTGTTACAGGAACCCTGCCAAAAGTACATCGAACAGGTGCAGTTTCTGGACATTCCTTGAAGCAATG TGGTCATCAAATAAGTGCTTGCCATCGAGACATTTATAAAGGAGTTGATATGAGAGGAGTCAATTTTAATGTGTCTAAGG TTAGCAGTGTTGAAGAATGCCAAAAAAGGTGCACCAATAACATTCGCTGCCAGTTTTTTTCATATGCCACGCAAACATTT CACAAGGCAGAGTACCGGAACAATTGCCTATTAAAGTACAGTCCCGGAGGAACACCTACCGCTATAAAGGTGCTGAGTAA CGTGGAATCTGGATTCTCACTGAAGCCCTGTGCCCTTTCAGAAATTGGTTGCCACATGAACATCTTCCAGCATCTTGCGT TCTCAGATGTGGATGTTGCCAGGTTTCTCACTCCAGATGCTTTTGTGTGTCGGACCATCTGCACCTATCACCCCAACTGC CTCTTCTTTACATTCTATACAAATGTATGGAAAATCGAGTCACAAAGAAATGTTTGTCTTCTTAAAACATCTGAAAGTGG CACACCAAGTTCCTCTACTCCTCAAGAAAACACCATATCTGGATATAGCCTTTTAACCTGCAAAAGAACTTTACCTGAAC CCTGCCATTCTAAAATTTACCCGGGAGTTGACTTTGGAGGAGAAGAATTGAATGTGACTTTTGTTAAAGGAGTGAATGTT TGCCAAGAGACTTGCACAAAGATGATTCGCTGTCAGTTTTTCACTTATTCTTTACTCCCAGAAGACTGTAAGGAAGAGAA GTGTAAGTGTTTCTTAAGATTATCTATGGATGGTTCTCCAACTAGGATTGCGTATGGGACACAAGGGAGCTCTGGTTACT CTTTGAGATTGTGTAACACTGGGGACAACGCTGTCTGCAACAAAAACAAGCACACGCATTGTTGGAGGAACAAACTCT TCTTGGGGAGAGTGGCCCTGGCAGGTGAGCCTGCAGGTGAAGCTGACAGCTCAGAGGCACCTGTGTGGAGGGTCACTCAT AGGACACCAGTGGGTCCTCACTGCTGCCCACTGCTTTGATGGGCTTCCCCTGCAGGATGTTGGCGCATCTATAGTGGCA TTTTAAATCTGTCAGACATTACAAAAGATACACCTTTCTCACAAATAAAAGAGATTATTATTCACCAAAACTATAAAGTC TCAGAAGGGAATCATGATATCGCCTTGATAAAACTCCAGGCTCCTTTGAATTACACTGAATTCCAAAAACCAATATGCCT ACCTTCCAAAGGTGACACAAGCACAATTTATACCAACTGTTGGGTAACCGGATGGGGCTTCTCGAAGGAGAAAGGTGAAA TCCAAAATATTCTACAAAAGGTAAATATTCCTTTGGTAACAAATGAAGAATGCCAGAAAAGATATCAAGATTATAAAATA ACCCAACGGATGGTCTGTGCTGGCTATAAAGAAGGGGGAAAAGATGCTTGTAAGGGAGATTCAGGTGGTCCCTTAGTTTG CAAACACAACGGAATGTGGCGTTTGGTGGGCATCACCAGCTGGGGTGAAGGCTGTGCCCGCAGGGAGCAACCTGGTGTCT ACACCAAAGTCGCTGAGTACATGGACTGGATTTTAGAGAAAACACAGAGCAGTGATGGAAAAGCTCAGATGCAGTCACCA GCATGAGAAGCAGTCCAGAGTCTAGGCAATTTTTACAACCTGAGTTCAAGTCAAATTCTGAGCCTGGGGGGTCCTCATCT GCAAAGCATGAAGAGTGGCATCTTCTTTGCATCCTAAG

A disclosed NOV3 protein (SEQ ID NO:30) encoded by SEQ ID NO:29 has 638

10   amino acid residues, and is presented using the one-letter code in Table 15B. Signal P, Psort

and/or Hydropathy results predict that NOV3 does have a signal peptide, and is likely to be

localized extracellularly with a certainty of 0.3700. In other embodiments NOV3 is also likely

to be localized to the lysosome (lumen) with a certainty of 0.1900, to the endoplasmic

reticulum (membrane) with a certainty of 0.1000, or to the endoplasmic reticulum (lumen)

15   with a certainty of 0.1000. The most likely cleavage site for NOV3 is between positions 19

and 20, (VSC-GC).

Table 15B. Encoded NOV3 protein sequence (SEQ ID NO:30).
MILFKQATYFISLFATVSCGCLTQLYENAFFRGGDVASMYTPNAQYCQMRCTFHPRCLLFSFLPASSINDMEKRFGCFLK DSVTGTLPKVHRTGAVSGHSLKQCGHQISACHRDIYKGVDMRGVNFNVSKVSSVEECQKRCTNNIRCQFFSYATQTFHKA EYRNNCLLKYSPGGTPTAIKVLSNVESGFSLKPCALSEIGCHMNIFQHLAFSDVDVARFLTPDAFVCRTICTYHPNCLFF TFYTNVWKIESQRNVCLLKTSESGTPSSSTPQENTISGYSLLTCKRTLPEPCHSKIYPGVDFGGEELNVTFVKGVNVCQE TCTKMIRCQFFTYSLLPEDCKEEKCKCFLRLSMDGSPTRIAYGTQGSSGYSLRLCNTGDNAVCTTKTSTRIVGGTNSSWG

```
EWPWQVSLQVKLTAQRHLCGGSLIGHQWVLTAAHCFDGLPLQDVWRIYSGILNLSDITKDTPFSQIKEIIIHQNYKVSEG
NHDIALIKLQAPLNYTEFQKPICLPSKGDTSTIYTNCWVTGWGFSKEKGEIQNILQKVNIPLVTNEECQKRYQDYKITQR
MVCAGYKEGGKDACKGDSGGPLVCKHNGMWRLVGITSWGEGCARREQPGVYTKVAEYMDWILEKTQSSDGKAQMQSPA
```

NOV3 is localized on chromosome 4 and is expressed in at least the following tissues: liver. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, and/or RACE sources.

NOV3 has homology to the amino acid sequences shown in the BLASTP data listed in Table 15C.

Table 15C. BLAST results for NOV3

Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
ref|NP_000883.1| (NM_000892)	plasma kallikrein B1 precursor	638	99	99	0.0
ref|XP_003474.2| (XM_003474)	plasma kallikrein B1 precursor [Homo sapiens]	638	98	98	0.0
dbj|BAA37147.1| (AB022425)	kallikrein [Sus scrofa]	643	79	89	0.0
ref|NP_032481.1| (NM_008455)	kallikrein B, plasma 1; kallikrein 3, plasma; antigen, prostate specific [Mus musculus]	638	76	86	0.0
ref|NP_036857 .1| (NM_012725)	plasma kallikrein [Rattus norvegicus]	638	74	85	0.0

The homology of these sequences is shown graphically in the ClustalW analysis shown in Table 15D.

Table 15D. ClustalW Analysis of NOV3

```
1)  NOV3a  (SEQ ID NO:30)
2)  ref|NP_000883.1|  (SEQ ID NO:111)
3)  ref|XP_003474.2|  (SEQ ID NO:112)
4)  dbj|BAA37147.1|   (SEQ ID NO:113)
5)  ref|NP_032481.1|  (SEQ ID NO:114)
6)  ref|NP_036857.1|  (SEQ ID NO:115)
```

```
                          10        20        30        40        50
                    ....|....|....|....|....|....|....|....|....|....|
NOV3        --------MILFKQATYFISLFATVSCGCLTQLYENAFFRGGDVASMYTP
```

96

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              ref|NP_000883.1|  --------MILFKQATYFISLFATVSCGCLTQLYENAFFRGGDVASMYTP
              ref|XP_003474.2|  --------MILFKQATYFISLFATVSCGCLTQLYENAFFRGGDVASMYTP
              dbj|BAA37147.1|   MEVIVLFRTISFRQAVYFMCLFAAVSCGCLPQLHKNTFFRGGDVSAMYTP
              ref|NP_032481.1|  --------MILFNRVGYFVSLFATVSCGCMTQLYKNTFFRGGDUAATYTP
   5          ref|NP_036857.1|  --------MILFKQVGYFVSLFATVSCGCLSQLYANTFFRGGDUAATYTP

                                         60        70        80        90       100
                                ....|....|....|....|....|....|....|....|....|....|
              NOV3              NAQYCQMRCTFHPRCLLFSFLPASSINDMEKRFGCFLKDSVTGTLPKVHR
   10         ref|NP_000883.1|  NAQYCQMRCTFHPRCLLFSFLPASSINDMEKRFGCFLKDSVTGTLPKVHR
              ref|XP_003474.2|  NAQYCQMRCTFHPRCLLFSFLPASSINDMEKRFGCFLKDSVTGTLPKVHR
              dbj|BAA37147.1|   SARECQMMCTFHPRCLLFSFLPADSTSVTDKRFGCFLKDSVTGMLPRVLR
              ref|NP_032481.1|  DAQYCQKMCTFHPRCLLFSFLAVTPPKETNKRFGCFMKESTTGTLPRTHR
   15         ref|NP_036857.1|  DAQHCQKMCTFHPRCLLFSFLAVSPTKETDKRFGCFMKESTTGTLPRTHR

                                        110       120       130       140       150
                                ....|....|....|....|....|....|....|....|....|....|
              NOV3              TGAVSGHSLKQCGHQISACHRDIYKGVDMRGVNFNVSKVSSVEECQKRCT
   20         ref|NP_000883.1|  TGAVSGHSLKQCGHQISACHRDIYKGVDMRGVNFNVSKVSSVEECQKRCT
              ref|XP_003474.2|  TGAVSGHSLKQCGHQISACHRDIYKGVDMRGVNFNVSKVSRVEECQKRCT
              dbj|BAA37147.1|   ENAISGHSLKQCGHQIRACHRDIYKGTDMRGVNFNVSKVKTVEECQERCT
              ref|NP_032481.1|  TGATSGHSLKQCGHQISACHRDIYKGLDMRGSNFNTSKTDNIEECQKLCT
              ref|NP_036857.1|  TGAISGHSLKQCGHQLSACHODIYEGLDMRGSNFNTSKTDSIEECQKLCT

                                        160       170       180       190       200
   25                           ....|....|....|....|....|....|....|....|....|....|
              NOV3              NNIRCQFFSYATQTFHKAEYRNNCLLKYSPGGTPTAIKVLSNVESGFSLK
              ref|NP_000883.1|  NNIRCQFFSYATQTFHKAEYRNNCLLKYSPGGTPTAIKVLSNVESGFSLK
              ref|XP_003474.2|  NNIRCQVFSYAPHTFHKXXXXNNCLLKYSPGGTPTAIKVLSNVESGFSLK
   30         dbj|BAA37147.1|   NSIHCLFFLYATQAFNNAEYRNNCLLKHSPGGTPTSIKVIANVESGFSLK
              ref|NP_032481.1|  NNFHCQFFTYATSAFYRPEYRKKCLLHSASGTPTSIKSADNLVSGFSLK
              ref|NP_036857.1|  NNIHCQFFLYATKAFHRPEYRKSCLLRSSSGTPTSIKPVDNLVSGFSLK

                                        210       220       230       240       250
   35                           ....|....|....|....|....|....|....|....|....|....|
              NOV3              PCALSEIGCHMNIFQHLAFSDVDVARFLTPDAFVCRTICTYHPNCLFFTF
              ref|NP_000883.1|  PCALSEIGCHMNIFQHLAFSDVDVARVLTPDAFVCRTICTYHPNCLFFTF
              ref|XP_003474.2|  PCALSEIGCHMNIFQHLAFSDVDVARVLTPDAFVCRTICTYHPNCLFFTF
   40         dbj|BAA37147.1|   PCADSEIGCHMDIFQHLAFSDVDVARVEAPDAFVCRTICTYHPNCLFFTF
              ref|NP_032481.1|  SCALSEIGCPMDIFQHSAFADLNVSQVITPDAFVCRTICTEHPNCLFFTF
              ref|NP_036857.1|  SCALSEIGCPMDIFQHFAFADLNVSQVMTPDAFVCRTVCTHHPNCLFFTF

                                        260       270       280       290       300
   45                           ....|....|....|....|....|....|....|....|....|....|
              NOV3              YTNVWKIESQRNVCLLKTSESGTPSSSTPQENTISGYSLLTCKRTLPEPC
              ref|NP_000883.1|  YTNVWKIESQRNVCLLKTSESGTPSSSTPQENTISGYSLLTCKRTLPEPC
              ref|XP_003474.2|  YTNVWKIESQRNVCLLKTSESGTPSSSTPQENTISGYSLLTCKRTLPEPC
              dbj|BAA37147.1|   YTNAWKIESQRNVCFLKTSHSGTPSFPTPQENAISGYSLLTCKQTLPEPC
   50         ref|NP_032481.1|  YTNEWETESQRNVCFLKTSKSGRPSPPIPQENAISGYSLLTCKRKTRPEPC
              ref|NP_036857.1|  YTNEWETESQRNVCFLKTSKSGRPSPPIIQENAVSGYSLFTCRKARPEPC

                                        310       320       330       340       350
                                ....|....|....|....|....|....|....|....|....|....|
   55         NOV3              HSKIYPGVDFGGEELNVTFVKGVNVCQETCTKMIRCQFFTYSLLPEDCKE
              ref|NP_000883.1|  HSKIYPGVDFGGEELNVTFVKGVNVCQETCTKMIRCQFFTYSLLPEDCKE
              ref|XP_003474.2|  HSKIYPGVDFGGEELNVTFVKGVNVCQETCTKMIRCQFFTYSLLPEDCKE
              dbj|BAA37147.1|   HSKIYSEVDFEGEELNVTFVQGANECQETCTKTIRCQFFTYSLHPEDCRG
   60         ref|NP_032481.1|  HSKIYSGVDFEGEELNVTFVQGADVCQETCTKTIRCQFFIYSLLPQDCKE
              ref|NP_036857.1|  HFKIYSCVAFEGEELNATFVQGADACQETCTKTIRCQFFTYSLLPQDCKA

                                        360       370       380       390       400
                                ....|....|....|....|....|....|....|....|....|....|
   65         NOV3              EKCKCFLRLSMDGSPTRIAYGTQGSSGYSLRLCNTGDNAVCTTKTSTRIV
              ref|NP_000883.1|  EKCKCFLRLSMDGSPTRIAYGTQGSSGYSLRLCNTGDNSVCTTKTSTRIV
              ref|XP_003474.2|  EKCKCFLRLSMDGSPTRIAYGTQGSSGYSLRLCNTGDNSVCTTKTSTRIV
              dbj|BAA37147.1|   EKCKCSLRLSSDGSPTKITEGMRASSGYSLRLCRSGDHSACATKANTRIV
   70         ref|NP_032481.1|  EGCKCSLRLSTDGSPTRITYGMQGSSGYSLRLCKLVDSPDCTTKINARIV
              ref|NP_036857.1|  EGCKCSLRLSTDGSPTRITYEAQGSSGYSLRLCKVVESSDCTTKINARIV

                                        410       420       430       440       450
                                ....|....|....|....|....|....|....|....|....|....|
              NOV3              GGTNSSWGEWPWQVSLQVKLTAQRHLCGGSLIGHQWVLTAAHCFDGLPLQ
              ref|NP_000883.1|  GGTNSSWGEWPWQVSLQVKLTAQRHLCGGSLIGHQWVLTAAHCFDGLPLQ
   75         ref|XP_003474.2|  GGTNSSWGEWPWQVSLQVKLTAQRHLCGGSLIGHQWVLTAAHCFDGLPLQ
              dbj|BAA37147.1|   GGTESFLGEWPWQVSLQAKLRAQNHLCGGSIIGHQWVLTAAHCFDGLSLP
              ref|NP_032481.1|  GGTNASLGEWPWQVSLQVKLVSQTHLCGGSIIGRQWVLTAAHCFDGIPYP
              ref|NP_036857.1|  GGTNSSLGEWPWQVSLQVKLVEQNHMCGGSIIGRQWILTAAHCFDGIPYP
```

```
                       460       470       480       490       500
                  ....|....|....|....|....|....|....|....|....|....|
NOV3              DVWRIYSGILNLSDITKDTPFSQIKEIIIHQNYKVSEGNHDIALIKLQAP
ref|NP_000883.1|  DVWRIYSGILNLSDITKDTPFSQIKEIIIHQNYKVSEGNHDIALIKLQAP
ref|XP_003474.2|  DVWRIYSGILNLSDITKDTPFSQIKEIIIHQNYKVSEGNHDIALIKLQAP
dbj|BAA37147.1|   DIWRIYGGILNISEITKETPFSQVKEIIIHQNYKILESGHDIALIKLETP
ref|NP_032481.1|  DVWRIYGGILSLSEITKETPSSSRIKEDIIHOEYKVSEGNDDIALIKLQTP
ref|NP_036857.1|  DVWRIYGGILNLSEITNKTPFSSIKEDIIHQKYKMSEGSYDIALIKLQTP

                       510       520       530       540       550
                  ....|....|....|....|....|....|....|....|....|....|
NOV3              LNYTEFQKPICLPSKGDTSTIYTNCWVTGWGFSKEKGEIQNILQKVNIPL
ref|NP_000883.1|  LNYTEFQKPICLPSKGDTSTIYTNCWVTGWGFSKEKGEIQNILQKVNIPL
ref|XP_003474.2|  LNYTEFQKPICLPSKGDTSTIYTNCWVTGWGFSKEKGEIQNILQKVNIPL
dbj|BAA37147.1|   LNYTDFQKPICLPSRDDTNVMYTNCWVTGWGFTEEKGEIQNILQKVNIPL
ref|NP_032481.1|  LNYTEFQKPICLPSKADTNTIYTNCWVTGWGMKEQGETQNILQKATIPL
ref|NP_036857.1|  LNYTEFQKPICLPSKADTNTIYTNCWVTGWGMKERGETQNILQKATIPL

                       560       570       580       590       600
                  ....|....|....|....|....|....|....|....|....|....|
NOV3              VINEECQKRYQDYKITQRMVCAGYKEGGKDACKGDSGGPLVCKHNGMWRL
ref|NP_000883.1|  VINEECQKRYQDYKITQRMVCAGYKEGGKDACKGDSGGPLVCKHNGMWRL
ref|XP_003474.2|  VINEECQKRYQDYKITQRMVCAGYKEGGKDACKGDSGGPLVCKHNGMWRL
dbj|BAA37147.1|   VSNEECQKSYRDHKISKMICAGYKEGGKDACKGESGGPLVCKNGIWHL
ref|NP_032481.1|  VPNEECQKKYRDYVLNKMICAGYKEGGTDACKGDSGGPLVCKHSGRWOL
ref|NP_036857.1|  VPNEECQKKYRDYVLTKMICAGYKEGGIDACKGDSGGPLVCKHSGRWQL

                       610       620       630       640
                  ....|....|....|....|....|....|....|....|....|.
NOV3              VGITSWGEGCARREQPGVYTKVAEYMDWILEKTQSSDGKAQMSPA
ref|NP_000883.1|  VGITSWGEGCARREQPGVYTKVAEYMDWILEKTQSSDGKAQMSPA
ref|XP_003474.2|  VGITSWGEGCARREQPGVYTKVAEYMDWILEDTQSSDGKAQMSPA
dbj|BAA37147.1|   VGITSWGEGCARREQPGVYTKVIEYMDWILEKTQDDDGQSWMK---
ref|NP_032481.1|  VGITSWGEGCGRKEQPGVYTKVSEYMDWILEKTQSSDVRALETSSA
ref|NP_036857.1|  VGITSWGEGCARKEQPGVYTKVAEYIDWILEKTQSSKERALETSPA
```

Tables 15E-G list the domain descriptions from DOMAIN analysis results against NOV3. This indicates that the NOV3a sequence has properties similar to those of other proteins known to contain this domain.

Table 15E Domain Analysis of NOV3

gnl|Smart|smart00020, Tryp_SPc, Trypsin-like serine protease CD-Length = 230 residues, 100.0% aligned   Score = 269 bits (687), Expect = 4e-73

Table 15F Domain Analysis of NOV3

gnl|Smart|smart00223, APPLE, APPLE domain CD-Length = 83 residues, 100.0% aligned   Score = 110 bits (276), Expect = 2e-25

Table 15G Domain Analysis of NOV3

gnl|Pfam|pfam00024, PAN, PAN domain CD-Length = 78 residues, 94.9% aligned Score = 44.3 bits (103), Expect = 2e-05

The human tissue kallikrein gene family was, until recently, thought to consist of only three genes. Two of these human kallikreins, prostate-specific antigen and human glandular

kallikrein 2, are currently used as valuable biomarkers of prostatic carcinoma. More recently, new kallikrein-like genes have been discovered. It is now clear that the human tissue kallikrein gene family contains at least 15 genes. All genes share important similarities, including mapping at the same chromosomal locus (19q13.4), significant homology at both the

5   nucleotide and protein level, and similar genomic organization. All genes encode for putative serine proteases and most of them are regulated by steroid hormones. Recent data suggest that at least a few of these kallikrein genes are connected to malignancy. (See Yousef et al., 2001, Endocr Rev 22:184).

The disclosed NOV3 nucleic acid of the invention encoding a Kallikrein-like protein

10   includes the nucleic acid whose sequence is provided in Table 15A or a fragment thereof. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 15A while still encoding a protein that maintains its Kallikrein-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just

15   described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least

20   in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject. In the mutant or variant nucleic acids, and their complements, up to about 0 percent of the bases may be so changed.

The disclosed NOV3 protein of the invention includes the Kallikrein-like protein

25   whose sequence is provided in Table 15B. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in Table 15B while still encoding a protein that maintains its Kallikrein-like activities and physiological functions, or a functional fragment thereof. In the mutant or variant protein, up to about 0 percent of the residues may be so changed.

30   The protein similarity information, expression pattern, and map location for the Kallikrein-like protein and nucleic acid (NOV3) disclosed herein suggest that NOV3 may have important structural and/or physiological functions characteristic of the kallikrein-like family. Therefore, the NOV3 nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications. These include serving as a specific or

selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a

5   nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration in vitro and in vivo.

The NOV3 nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications implicated in various diseases and disorders described below. For example, the compositions of the present invention will have efficacy for treatment of patients

10   suffering from cancer, particularly prostate cancer, metabolic disorders, heart disease, hypertension, and/or other pathologies..

NOV3 nucleic acids and polypeptides are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the

15   art, using prediction from hydrophobicity charts, as described in the "Anti-NOVX Antibodies" section below. For example the disclosed NOV3 protein have multiple hydrophilic regions, each of which can be used as an immunogen. This novel protein also has value in development of powerful assay system for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug

20   targets for various disorders.

NOV4

A disclosed NOV4 nucleic acid of 1094 nucleotides identified as SEQ ID NO:31 (designated CuraGen Acc. No. CG56166-01) encoding a novel Multidrug transporter-like

25   protein is shown in Table 16A.

Table 16A. NOV4 Nucleotide Sequence (SEQ ID NO:31)
TCTTCCACCTTTCTCCATTCCTCTAGGTGCTTTTTCTGAACCTGGATGTGAGGCATTAAAGGATCCGACGGAAATAGAAT TGAAGGCATTCTAAAATGGCTAACCGTACAGTGAAGGATGCGCACAGCATCCATGGCACCAACCCTCAATATCTGGTGGA GAAGATCATTCGAACGCGAATCTATGAGTCCAAGTACTGGAAAGAGGAGTGCTTTGGACTTACAGCTGAACTTGTAGTCG ATAAAGCCATGGAGTTAAGGTTTGTGGGTGGCGTCTATGGTGGCAACATAAAACCAACACCCTTTCTGTGTTTAACCTTG AAGATGCTTCAAATTCAACCCGAGAAGGATATCATTGTAGAGTTTATCAAAAATGAAGATTTCAAGTATGTCCGCATGCT GGGGGCACTTTACATGAGGCTGACAGGCACTGCAATTGATTGCTACAAGTACTTGGAACCTTTGTACAATGACTATCGAA AAATCAAGAGCCAGAACCGAAATGGGGAGTTTGAATTGATGCATGTTGATGAGTTTATTGATGAACTATTGCAAAGTGAG AGAGTCTGTGATATCATTCTGCCCCGACTACAGAAACGCTATGTATTAGAGGAAGCTGAGCAACTGGAGCCTCGAGTTAG TGCTCTGGAAGAGGACATGGATGATGTGGAGTCCAGTGAAGAGGAAGAAGAGGAGGATGAGAAGTTGGAAAGAGTGCCAT CACCTGATCACCGCCGGAGAAGCTACCGAGACTTGGACAAGCCCCGTCGCTCTCCCACACTGCGCTACAGGAGGAGTAGG AGCCGGTCTCCCAGAAGGCGGAGTCGATCTCCCAAAAGGAGAAGCCCCTCCCCTCGCCGAGAAAGGCATCGGAGCAAGAG TCCAAGACGTCACCGCCAGCAGGTCCCGAGATCGGCGGCACAGATCCCGTTCCAAGTCCCCAGGTCATCACCGTAGTCACA GACACAGGAGCCACTCAAAGTCTCCCGAAAGGTCTAAGAAGAGCCACAAGAAGAGCCGGAGAGGGAATGAGTAATGGACT CAGTTTGGTTTTAGTCCACATGGCCTCCTGTGGATATAAGGATATCTGTATGTG

A NOV4 polypeptide (SEQ ID NO:32) encoded by SEQ ID NO:31 is 312 amino acid residues and is presented using the one letter code in Table 16B. Signal P, Psort and/or Hydropathy results predict that NOV4 has no signal peptide and is likely to be localized at the nucleus with a certainty of 0.9894. In other embodiments, NOV4 may also be localized to the mitochondrial matrix space with a certainty of 0.1000, or the lysosome (lumen) with a certainty of 0.1000.

Table 16B. NOV4 protein sequence (SEQ ID NO:32)
MANRTVKDAHSIHGTNPQYLVEKIIRTRIYESKYWKEECFGLTAELVVDKAMELRFVGGVYGGNIKPTPFLCLTLKMLQI QPEKDIIVEFIKNEDFKYVRMLGALYMRLTGTAIDCYKYLEPLYNDYRKIKSQNRNGEFELMHVDEFIDELLQSERVCDI ILPRLQKRYVLEEAEQLEPRVSALEEDMDDVESSEEEEEEDEKLERVPSPDHRRRSYRDLDKPRRSPTLRYRRSRSRSPR RRSRSPKRRSPSPRRERHRSKSPRRHRSRSRDRRHRSRSKSPGHHRSHRHRSHSKSPERSKKSHKKSRRGNE

NOV4 is expressed in at least multiple normal and cancerous tissues.

NOV4 has homology to the amino acid sequences shown in the BLASTP data listed in Table 16C.

Table 16C. BLAST results for NOV4					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi|10727696|gb|AAF5 8976.2| (AE003834)	CG8054 gene product [Drosophila melanogaster]	856	57	70	2e-90
gi|17454329|ref|XP 061203.1| (XM_061203)	similar to CG8054 gene product (H. sapiens)	140	86	88	6e-66
gi|14249602|ref|NP 116253.1| (NM_032864)	hypothetical protein FLJ14936 [Homo sapiens]	236	99	99	2e-65
gi|17559118|ref|NP 505762.1| (NM_073361)	D1054.14.p [Caenorhabditis elegans]	320	62	79	3e-62
gi|15226730|ref|NP 181597.1| (NC_003071)	hypothetical protein [Arabidopsis thaliana]	363	63	7	4e-61

The homology of these sequences is shown graphically in the ClustalW analysis shown in Table 16D.

Table 16D ClustalW Analysis of NOV4

```
1)  NOV4 (SEQ ID NO: 32)
2)  gi|10727696|  (SEQ ID NO:116)
3)  gi|17454329|  (SEQ ID NO:117)
4)  gi|14249602|  (SEQ ID NO:118)
5)  gi|17559118|  (SEQ ID NO:119)
6)  gi|15226730|  (SEQ ID NO:120)
```

```
                           10        20        30        40        50
                   ....|....|....|....|....|....|....|....|....|....|
          NOV4     MANRTVKDAHSIHGTNPQXLVEKIIRTRIYESKXWKEECFGLTAELXVDK
          gi|10727696| MANRTVKEAKNVHGTNPQXLIEKIIRSRIYDSKXWKDQCFALTAELXVDK
          gi|17454329| MANRTLKDAHSVRGTNPQXLVGKIIRMRICESKXWKEECFGLMAELXVDN
          gi|14249602| MLQIQPEKDIIVEFIKNGDFKYVRXLGAEYMRLTGTAIDCYKYLEPLYND
          gi|17559118| MANRTEKAAKTVKGTNPQXLVEKIIRQRIYDSMXWKEHCFALTAELXVDK
          gi|15226730| MANRTDPLAKNIRGTNPQNLVEKIXRTKIYQHTXWKEQCFGLTAEXLVDK

                           60        70        80        90        100
                   ....|....|....|....|....|....|....|....|....|....|
          NOV4     AMELRXVGGVYGGNIKPTPFLCLTLKMLQIQPEKDIIVEFIKNEDFKYVR
          gi|10727696| AMELRXVGGVYGGNIKPTQFLCLTLKMLQIQPEKDIVVEFIKNEDFKYVR
          gi|17454329| AMELMXVGGEYGGNIKPTPFLCLILKMLQIQSEKGITAEFIENEDFKYVH
          gi|14249602| YRKXXSQNRNGEFEXMHVDEFIDELLHSERVCDIILPRLQKRYVLEEAEQ
          gi|17559118| GMDLRXIGGIXAGNIKPTPFLCIALKMLQIQPDKDIVXBFIQEEFKYXR
          gi|15226730| AMELDHXGGTXGGSRKPTPFLCLILKMLQIQPEKEIIVVEFIKNDDKYVR

                           110       120       130       140       150
                   ....|....|....|....|....|....|....|....|....|....|
          NOV4     XLGAXYMRLTGTAIDCYKYLEPLYNDXRKIXSQNRNGEFELMHVDEFIDE
          gi|10727696| ALGAFYXRLTGAAXDCYKYLEPLYIDNRKXRRONRACQFEIVYMDEYIDE
          gi|17454329| XLGAXYXRLMGTAIDCYKYLEPLYNDXRKIXSQNRNGGLN
          gi|14249602| XEPRVSALEEDMDDVESSEEXEEEDXEKLERVPSPDHRRRSYRDLDKPRRS
          gi|17559118| ALGAMYXRLTFDSTXIYKYLEPLYNDXRKXRYMXKMGRFEAIYMDDFIDN
          gi|15226730| XLGAFYXRLTGTDVDVXRYLEPLYNDXRKXRQXLSDGNLFLWFGIEFSLT

                           160       170       180       190       200
                   ....|....|....|....|....|....|....|....|....|....|
          NOV4     LLQSERVCDIILPRLQKRYVLEEAEQLEPRVSALEEDMDDVESSEEEEEE
          gi|10727696| LLRNDRVCDIILPRIQKRSILEENNEIEPKVSVLDEDLDDELPSDEEKAD
          gi|17454329|
          gi|14249602| PTLRYRRSRSRSPRRRSRSPKRRSPSPRRERHRSKSPRRHRSRSRDRRHR
          gi|17559118| LLREDRYCDIQLPRLQKRWALEEVDMLPSYKSLLDGDLVAMSDSDSEEEE
          gi|15226730| HVDEVIEELLTKDYSCDIAMPRLKKRWTLEQNGLLEPRKSVLEDDFEEEE

                           210       220       230       240       250
                   ....|....|....|....|....|....|....|....|....|....|
          NOV4     DEKLERVPSPDHRRRSYRDLDKPRRSPTLRYRRSRSRSPRRRSRSPKRRS
          gi|10727696| ETNRPKENSTAVRRPRRVRSKSRSRSRERERRSGQGNSARSRDYYDELED
          gi|17454329|
          gi|14249602| SRSKSPGHHRSHRHRSHSKSPERSKKSHKKSRRGNE
          gi|17559118| VTKKEKPRLTSRRRSRSRDRERDVGDRREVREREKLKERRERGDDEPGPS
          gi|15226730| EKEENEGIADGSEDEMDQRRKSPERERERDRDRRRDSHRHRDRDYDRDYD

                           260       270       280       290       300
                   ....|....|....|....|....|....|....|....|....|....|
          NOV4     PSPRRERHRSKSPRRHRSRSRDRRHRSRSKSPGHHRSHRHRSHSKSPERS
          gi|10727696| YDRQRNRVRNRDTHNEDYDRRQNNGRHDRERERQDRDSIRERERDGDRDR
          gi|17454329|
          gi|14249602|
          gi|17559118| SSGSGRRDDRDDRRRDRDRSRDRDRRDRRDDDRRDKKKESRRGGADNDEE
          gi|15226730| MDRDHDRDYERERGHGRDRDRERDRDHYERDRDRERGRDRERDRRDRAR

                           310       320       330       340       350
                   ....|....|....|....|....|....|....|....|....|....|
          NOV4     KKSHKKSRRGNE
          gi|10727696| RDRERERERDRGRHDQRERDSRGEHWCKPDASTSSVLDSEQVGTERNGSM
          gi|17454329|
          gi|14249602|
          gi|17559118| REIAEANALRAKLGLAPLER
          gi|15226730| RRSRSRSRDRKRHETDDVRDREEPKKKKEKKEKMKEDGTDHPNPEIAEMN

                           360       370       380       390       400
                   ....|....|....|....|....|....|....|....|....|....|
          NOV4
          gi|10727696| PRSDTEALVADVETGEDSAPRILDASSAASSEQVDPPPVPPPHDYSSYRW
          gi|17454329|
          gi|14249602|
          gi|17559118| RLRASLGMKPLRD
          gi|15226730|

                           410       420       430       440       450
                   ....|....|....|....|....|....|....|....|....|....|
          NOV4
          gi|10727696| FILEPAVFLIFFARNLIGAVYQNQILYQTCITIEKFNATQCEPLLGIDRG
          gi|17454329|
```

Numbers in left margin: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75

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         gi|14249602|
         gi|17559118|
         gi|15226730|

                         460       470       480       490       500
                    ....|....|....|....|....|....|....|....|....|....|
         NOV4
         gi|10727696|  SDADKEVEVIVQTYSANIMMTTSLLESIIPAFASLFLGPWSDKFGRRPIL
         gi|17454329|
         gi|14249602|
         gi|17559118|
         gi|15226730|

                         510       520       530       540       550
                    ....|....|....|....|....|....|....|....|....|....|
         NOV4
         gi|10727696|  LTTFTGYLTGALILIVITYITRSTNISPWWFLLSSVPSVVSGGTCALITG
         gi|17454329|
         gi|14249602|
         gi|17559118|
         gi|15226730|

                         560       570       580       590       600
                    ....|....|....|....|....|....|....|....|....|....|
         NOV4
         gi|10727696|  IYCYISDVAKERKKALRMVLNEASLCAGIMVGNVASGYIYAATNALVLFS
         gi|17454329|
         gi|14249602|
         gi|17559118|
         gi|15226730|

                         610       620       630       640       650
                    ....|....|....|....|....|....|....|....|....|....|
         NOV4
         gi|10727696|  IAGSLMMFALMYVLLFVPESLNPGDIHTGSRVREFFRFDLVTDLIRTCFK
         gi|17454329|
         gi|14249602|
         gi|17559118|
         gi|15226730|

                         660       670       680       690       700
                    ....|....|....|....|....|....|....|....|....|....|
         NOV4
         gi|10727696|  RRPNFDRTIIWLTMIALTIAIFDMEGESTVNYMFVQDKFNWTIKDFSLFN
         gi|17454329|
         gi|14249602|
         gi|17559118|
         gi|15226730|

                         710       720       730       740       750
                    ....|....|....|....|....|....|....|....|....|....|
         NOV4
         gi|10727696|  ASRIVIQIVGSIVGMLVLRRVLKMSIVTMAMLSLACCVLESTVRATAVYW
         gi|17454329|
         gi|14249602|
         gi|17559118|
         gi|15226730|

                         760       770       780       790       800
                    ....|....|....|....|....|....|....|....|....|....|
         NOV4
         gi|10727696|  QELYLGMTLGMMRGVMGPMCRAILSHVAPATEVGKIFALTTSMESVSPLG
         gi|17454329|
         gi|14249602|
         gi|17559118|
         gi|15226730|

                         810       820       830       840       850
                    ....|....|....|....|....|....|....|....|....|....|
         NOV4
         gi|10727696|  AAPLYTTVYKATLENYPGAFNFISAALYFVCYILIAVIFGIQKSMGSSSV
         gi|17454329|
         gi|14249602|
         gi|17559118|
         gi|15226730|


                    ....|.
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```
NOV4
gi|10727696|    YQAIGS
gi|17454329|
gi|14249602|
gi|17559118|
gi|15226730|
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The development of refractory disease in acute myeloid or lymphoblastic leukaemias (AML, ALL) and multiple myeloma (MM) is frequently associated with the expression of one or several multidrug resistance (MDR) genes. MDR1, MRP1 and LRP have been identified as important adverse prognostic factors in AML, T-ALL and MM. Recently, it has become possible to reverse clinical multidrug resistance by blocking P-glycoprotein-mediated drug efflux. (See Sonneveld, 2000, J. Intern Med, 247:521).

A key issue in the treatment of acute leukemia is the development of resistance to chemotherapeutic drugs. Several mechanisms may account for this phenomenon, including failure of the cell to undergo apoptosis in response to chemotherapy, or failure of the drug to reach and/or affect its intracellular target. This review focuses on the latter mechanism, and on intracellular drug transport resistance mechanisms in particular. Expression of the ATP-binding cassette (ABC) transporter P-glycoprotein (Pgp) has generally been reported to correlate with prognosis in acute myeloid leukemia (AML). Additionally, but more controversial, expression of the ABC transporter multidrug resistance protein (MRP) and the vault-transporter lung resistance protein (LRP) have been correlated with outcome in AML. Despite these findings, functional efflux assays indicate the presence of non-Pgp, non-MRP transporters in AML. Recently, a novel ABC transporter, breast cancer resistance protein (BCRP) was cloned and sequenced in our laboratory. Transfection and overexpression of BCRP in drug-sensitive cells confers drug-resistance to the cells. BCRP is a half-transporter, and may homodimerize or form heterodimers (with a yet unknown half-transporter) to produce an active transport complex. Relatively high expression of BCRP mRNA is observed in approximately 30% of AML cases, suggesting a potential role for this new transporter in drug resistance in leukemia. (See Ross, 2000, Leukemia 14:467).

The disclosed NOV4 nucleic acid of the invention encoding a Multidrug transporter - like protein includes the nucleic acid whose sequence is provided in Table 16A or a fragment thereof. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 16A while still encoding a protein that maintains its Multidrug transporter -like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are

complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or

5   derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject. In the mutant or variant nucleic acids, and their complements, up to about 42 percent of the bases may be so changed.

The disclosed NOV4 protein of the invention includes the Multidrug transporter -like

10   protein whose sequence is provided in Table 16B. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in Table 16B while still encoding a protein that maintains its Multidrug transporter -like activities and physiological functions, or a functional fragment thereof. In the mutant or variant protein, up to about 42 percent of the residues may be so changed.

15   The protein similarity information, expression pattern, and map location for the Multidrug transporter-like protein and nucleic acid (NOV4) disclosed herein suggest that this NOV4 protein may have important structural and/or physiological functions characteristic of the Multidrug transporter family. Therefore, the NOV4 nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications. These include

20   serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a

25   composition promoting tissue regeneration in vitro and in vivo.

The NOV4 nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications implicated in various diseases and disorders described below. For example, the compositions of the present invention will have efficacy for treatment of patients suffering from diabetes, fetal growth retardation, cancer, glycogen storage disease,

30   hypertension and/or other disorders and conditions. The NOV4 nucleic acids, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

NOV4 nucleic acids and polypeptides are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or

diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-NOVX Antibodies" section below. These novel proteins can be used in assay systems for functional analysis of various human disorders, which will help in understanding of pathology of the disease and

5   development of new drug targets for various disorders.

NOV5

A disclosed NOV5 nucleic acid of 3168 nucleotides identified as SEQ ID NO:33 (also referred to as CG56151-01) encoding a novel glucose transporter type 2-like protein is shown

10   in Table 17A.

<table>
<tr><td colspan="1">Table 17A. NOV5 Nucleotide Sequence (SEQ ID NO:33)</td></tr>
<tr><td>
CACAAGACCTGGAATTGACAGGACTCCCAACTAGTACAATGACAGAAGATAAGGTCACTGGGACCCTGGTTTTCACTGTC
ATCACTGCTGTGCTGGGTTCCTTCCAGTTTGGATATGACATTGGTGTGATCAATGCACCTCAACAGGTAATAATATCTCA
CTATAGACATGTTTTGGGTGTTCCACTGGATGACCGAAAAGCTATCAACAACTATGTTATCAACAGTACAGATGAACTGC
CCACAATCTCATACTCAATGAACCCAAAACCAACCCCTTGGGCTGAGGAAGAGACTGTGGCAGCTGCTCAACTAATCACC
ATGCTCTGGTCCCTGTCTGTATCCAGCTTTGCAGTTGGTGGAATGACTGCATCATTCTTTGGTGGGTGGCTTGGGGACAC
ACTTGGAAGAATCAAAGCCATGTTAGTAGCAAACATTCTGTCATTAGTTGGAGCTCTCTTGATGGGGTTTTCAAAATTGG
GACCATCTCATATACTTATAATTGCTGGAAGAAGCATATCAGGACTATATTGTGGGCTAATTTCAGGCCTGGTTCCTATG
TATATCGGTGAAATTGCTCCAACCGCTCTCAGGGGAGCACTTGGCACTTTTCATCAGCTGGCCATCGTCACGGGCATTCT
TATTAGTCAGATTATTGGTCTTGAATTTATCTTGGGCAATTATGATCTGTGGCACATCCTGCTTGGCCTGTCTGGTGTGC
GAGCCATCCTTCAGTCTCTGCTACTCTTTTTCTGTCCAGAAGCCCCAGATACCTTTACATCAAGTTAGATGAGGAAGTC
AAAGCAAAACAAAGCTTGAAAAGACTCAGAGGATATGATGATGTCACCAAAGATATTAATGAAATGAGAAAAGAAAGAGA
AGAAGCATCGAGTGAGCAGAAAGTCTCTATAATTCAGCTCTTCACCAATTCCAGCTACCGACAGCCTATTCTAGTGGCAC
TGATGCTGCATGTGGCTCAGCAATTTTCCGGAATCAATGGCATTTTTTACTACTCAACCAGCATTTTTCAGACGGCTGGT
ATCAGCAACCTGTTTATGCAACCATTGGAGTTGGCGCTGTAAACATGGTTTTCACTGCTGTCTCTGTATTCCTTGTGGA
GAAGGCAGGGCGACGTTCTCTCTTTCTAATTGGAATGAGTGGGATGTTTGTTTGTGCCATCTTCATGTCAGTGGGACTTG
TGCTGCTGAATAAGTTCTCTTGGATGAGTTATGTGAGCATGATAGCCATCTTCCTCTTTGTCAGCTTCTTTGAAATTGGG
CCAGGCCCGATCCCCTGGTTCATGGTGGCTGAGTTTTTCAGTCAAGGACCACGTCCTGCTGCTTTAGCAATAGCTGCATT
CAGCAATTGGACCTGCAATTTCATTGTAGCTCTGTGTTTCCAGTACATTGCGGACTTCTGTGGACCTTATGTGTTTTTCC
TCTTTGCTGGAGTGCTCCTGGCCTTTACCCTGTTCACATTTTTTAAAGTTCCAGAAACCAAAGGAAAGTCTTTTGAGGAA
ATTGCTGCAGAATTCCAAAAGAAGAGTGGCTCAGCCCACAGGCCAAAAGCTGCTGTAGAAATGAAATTCCTAGGAGCTAC
AGAGACTGTGTAAAAAAAAAAACCCTGCTTTTTGACATGAACAGAAACAATAAGGGAACCGTCGTTTTTAAATGATGATT
CCTTGAGCATTTTATATCCACATCTTTAAGTATTGTTTTATTTTTATGTGCTCTCATCAGAAATGTCATCAAATATTACC
AAAAAAGTATTTTTTAAGTTAGAGAATATATTTTTGATGGTAAGACTGTAATTAAGTAAACCAAAAAGGCTAGTTTATT
TTGTTACACTAAAGGGCAGGTGGTTCTAATATTTTTAGCTCTGTTCTTTATAACAAGGTTCTTCTAAAATTGAAGAGATT
TCAACATATCATTTTTTTAACACATAACTAGAAACCTGAGGATGCAACAAATATTTATATATTTGAATATCATTAAATTG
GAATTTTCTTACCCATATATCTTATGTTAAAGGAGATATGGCTAGTGGCAATAAGTTCCATGTTAAAATAGACAACTCTT
CCATTTATTGCACTCAGCTTTTTTCTTGAGTACTAGAATTTGTATTTTGCTTAAAATTTTACTTTTGTTCTGTATTTTCA
TGTGGAATGGATTATAGAGTATACTAAAAAATGTCTATAGAGAAAAACTTTCATTTTTGGTAGGCTTATCAAAATCTTTC
AGCACTCAGAAAAGAAAACCATTTTAGTTCCTTTATTTAATGGCCAAATGGTTTTTGCAAGATTTAACACTAAAAAGGTT
TCACCTGATCATATAGCGTGGGTTATCAGTTAACATTAACATCTATTATAAAACCATGTTGATTCCCTTCTGGTACAATC
CTTTGAGTTATAGTTTGCTTTGCTTTTTAATTGAGGACAGCCTGGTTTTCACATACACTCAAACAATCATGAGTCAGACA
TTTGGTATATTACCTCAAATTCCTAATAAGTTTGATCAAATCCTAATAAGTTGAAGAAAATTTGAAGTAAAGGATTGATCACTTT
GTTAAAAATATTTTCTGAATTATTATGTCTCAAAATAAGTTGAAAAGGTAGGGTTTGAGGATTCCTGAGTGTGGGCTTCT
GAAACTTCATAAATGTTCAGCTTCAGACTTTTATCAAAATCCCTATTTAATTTTCCTGGAAAGACTGATTGTTTTATGGT
GTGTTCCTAACATAAAATAATCGTCTCCTTTGACATTTCCTTCTTTGTCTTAGCTGTATACAGATTCTAGCCAAACTATT
CTATGGCCATTACTAACACGCATTGTACACTATCTATCTGCCTTTACCTACATAGGCAAATTGGAAATACACAGATGATT
AAACAGACTTTAGCTTACAGTCAATTTTACAATTATGGAAATATAGTTCTGATGGGTCCCAAAAGCTTAGCAGGGTGCTA
ACGTATCTCTAGGCTGTTTTCTCCACCAACTGGAGCACTGATCAATCCTTCTTATGTTTGCTTTAATGTGTATTGAAGAA
AAGCACTTTTTAAAAAGTACTCTTTAAGAGTGAAATAATTAAAAACCACTGAACATTTGCTTTGTTTTCTAAAGTTGTTC
ACATATATGTAATTTAGCAGTCCAAAGAACAAGAAATTGTTTCTTTTC
</td></tr>
</table>

The NOV5 nucleic acid was identified on chromosome 3.

A disclosed NOV5 polypeptide (SEQ ID NO:34) encoded by SEQ ID NO:33 is 524 amino acid residues and is presented using the one-letter code in Table 17B. Signal P, Psort

15   and/or Hydropathy results predict that NOV5 has a signal peptide and is likely to be localized

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in the plasma membrane with a certainty of 06400. In other embodiments, NOV5 may also be localized to the golgi body with a certainty of 0.4600, the endoplasmic reticulum (membrane) with a certainty of 0.3700, or the endoplasmic reticulum (lumen) with a certainty of 0.1000. The most likely cleavage site is between positions 20 and 21:VGL-SF.

Table 17B. Encoded NOV5 protein sequence (SEQ ID NO:34)
MTEDKVTGTLVFTVITAVLGSFQFGYDIGVINAPQQVIISHYRHVLGVPLDDRKAINNYVINSTDELPTISYSMNPKPTP WAEEETVAAAQLITMLWSLSVSSFAVGGMTASFFGGWLGDTLGRIKAMLVANILSLVGALLMGFSKLGPSHILIIAGRSI SGLYCGLISGLVPMYIGEIAPTALRGALGTFHQLAIVTGILISQIIGLEFILGNYDLWHILLGLSGVRAILQSLLLFFCP ESPRYLYIKLDEEVKAKQSLKRLRGYDDVTKDINEMRKEREEASSEQKVSIIQLFTNSSYRQPILVALMLHVAQQFSGIN GIFYYSTSIFQTAGISKPVYATIGVGAVNMVFTAVSVFLVEKAGRRSLFLIGMSGMFVCAIFMSVGLVLLNKFSWMSYVS MIAIFLFVSFFEIGPGPIPWFMVAEFFSQGPRPAALAIAAFSNWTCNFIVALCFQYIADFCGPYVFFLFAGVLLAFTLFT FFKVPETKGKSFEEIAAEFQKKSGSAHRPKAAVEMKFLGATETV

NOV5 is expressed in at least the liver. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources.

NOV5 has homology to the amino acid sequences shown in the BLASTP data listed in Table 17C.

Table 17C. BLAST results for NOV5					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi|4557851|ref|NP_0 00331.1| (NM_000340)	solute carrier family 2 (facilitated glucose transporter), member 2 [Homo sapiens]	524	100	100	0.0
gi|12836740|dbj|BAB 23792.1|	(AK005068) putative [Mus musculus]	523	81	89	0.0
gi|90517|pir||S0531 9	glucose transport protein, hepatic - mouse	523	81	89	0.0
gi|2143756|pir||S68 362	glucose transport protein type 2 - rat	522	81	89	0.0
gi|92281|pir||A3155 6	glucose transport protein, hepatic - rat	522	81	89	0.0

The homology of these sequences is shown graphically in the ClustalW analysis shown in Table 17D.

Table 17D Clustal W Sequence Alignment

1) NOV5 (SEQ ID NO: 34)
2) gi|4557851| (SEQ ID NO:121)
3) gi|17380402| (SEQ ID NO:122)

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4)  gi|13654262|  (SEQ ID NO:123)
5)  gi|2143756|   (SEQ ID NO:124)
6)  gi|6981548|   (SEQ ID NO:125)
```

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                    10        20        30        40        50
             ....|....|....|....|....|....|....|....|....|....|
NOV5         MEDKMTGTLVFTVITAVLGSFQFGYDIGVINAPQOVIISHYRHVLGVPL
gi|4557851|  MEDKVTGTLVFTVITAVLGSFQFGYDIGVINAPQOVIISHYRHVLGVPL
gi|17380402| MSEDKITGTLAFTVFTAVLSSFQFGYDIGVINAPQEVIISHYRHVLGVPL
gi|13654262| MSEDKITGTLAFTVFTAVLSSFQFGYDIGVINAPQEVIISHYRHVLGVPL
gi|2143756|  MSEDKITGTLAFTVFTAVLGSFQFGYDIGVINAPQEVIISHYRHVLGVPL
gi|6981548|  MSEDKITGTLAFTVFTAVLGSFQFGYDIGVINAPQEVIISHYRHVLGVPL

                    60        70        80        90        100
             ....|....|....|....|....|....|....|....|....|....|
NOV5         DDRKAINNYVINSTDELPTTSYSMNPKPTPWAEEETVAAAQLITMLWSLS
gi|4557851|  DDRKAINNYVINSTDELPTTSYSMNPKPTPWAEEETVAAAQLITMLWSLS
gi|17380402| DDRKAAINYDVNGTDTPLTVTPAYT-TPAPWDEEETEGSAHIVTMLWSLS
gi|13654262| DDRKAAINYDVNGTDTPLTVTPAYT-TPAPWDEEETEGSAHIVTMLWSLS
gi|2143756|  DDRRATINYDINGTDTPLIVTPAHT-TPDAWEEEETEGSAHIVTMLWSLS
gi|6981548|  DDRRATINYDINGTDTPLIVTPAHT-TPDAWEEEETEGSAHIVTMLWSLS

                    110       120       130       140       150
             ....|....|....|....|....|....|....|....|....|....|
NOV5         VSSFAVGGMTASFFGGWLGDTLGRIKAMLVANILSLVGALLMGFSKLGPS
gi|4557851|  VSSFAVGGMTASFFGGWLGDTLGRIKAMLVANILSLVGALLMGFSKLGPS
gi|17380402| VSSFAVGGMVASFFGGWLGDKLGRIKAMLAANSLSLTGALLMGCSKFGPA
gi|13654262| VSSFAVDGMVASFFGGWLGDKLGRIKAMLAANSLSLTGALLMGCSKFGPA
gi|2143756|  VSSFAVGGMVASFFGGWLGDKLGRIKAMLAANSLSLTGALLMGCSKFGPA
gi|6981548|  VSSFAVGGMVASFFGGWLGDKLGRIKAMLAANSLSLTGALLMGCSKFGPA

                    160       170       180       190       200
             ....|....|....|....|....|....|....|....|....|....|
NOV5         HILIIAGRSISGLYCGLISGLVPMYIGEIAPTALRGALGTFHQLAIVTGI
gi|4557851|  HILIIAGRSISGLYCGLISGLVPMYIGEIAPTALRGALGTFHQLAIVTGI
gi|17380402| HALIIAGRSVSGLYCGLISGLVPMYIGEIAPTTLRGALGTLHQLALVTGI
gi|13654262| HALIIAGRSVSGLYCGLISGLVPMYIGEIAPTTLRGALGTLHQLALVTGI
gi|2143756|  HALIIAGRSVSGLYCGLISGLVPMYIGEIAPTTLRGALGTLHQLALVTGI
gi|6981548|  HALIIAGRSVSGLYCGLISGLVPMYIGEIAPTTLRGALGTLHQLALVTGI

                    210       220       230       240       250
             ....|....|....|....|....|....|....|....|....|....|
NOV5         LISQIIGLEFILGNYDLWHILLGLSGVRAILQSLLLFCPESPRYLYIKL
gi|4557851|  LISQIIGLEFILGNYDLWHILLGLSGVRAILQSLLLFCPESPRYLYIKL
gi|17380402| LISQIAGLSFILGNQDHWHILLGLSAVPALLQCLLLLFCPESPRYLYIKL
gi|13654262| LISQIAGLSFILGNQDHWHILLGLSAVPALLQCLLLLFCPESPRYLYIKL
gi|2143756|  LISQIAGLSFILGNQDYWHILLGLSAVPALLQCLLLLFCPESPRYLYIKL
gi|6981548|  LISQIAGLSFILGNQDYWHILLGLSAVPALLQCLLLLFCPESPRYLYNL

                    260       270       280       290       300
             ....|....|....|....|....|....|....|....|....|....|
NOV5         DEEVKAKOSLKRLRGYDDVTKDINEMRKBREEASSEQKVSIIQLFTNSSY
gi|4557851|  DEEVKAKOSLKRLRGYEDVTKDINEMRKBREEASSEQKVSIIQLFTNSSY
gi|17380402| EEEVRAKKSLKRLRGTEDVTKDINEMKKEKEEASTEQKVSVIQLFTDANY
gi|13654262| EEEVRAKKSLKRLRGTEDVTKDINEMKKEKEEASTEQKVSVIQLFTDANY
gi|2143756|  EEEVRAKKSLKRLRGTEDITKDINEMRKEKEEASTEQKVSVIQLFTDPNY
gi|6981548|  EEEVRAKKSLKRLRGTEDITKDINEMRKEKEEASTEQKVSVIQLFTDPNY

                    310       320       330       340       350
             ....|....|....|....|....|....|....|....|....|....|
NOV5         RQPILVALMLHVAQQFSGINGIFYYSTSIFQTAGISKPVYATIGVGAVNM
gi|4557851|  RQPILVALMLHVAQQFSGINGIFYYSTSIFQTAGISKPVYATIGVGAVNM
gi|17380402| RQPILVALMLHVAQQFSGINGIFYYSTSIFQTAGISQPVYATIGVGAINM
gi|13654262| RQPILVALMLHVAQQFSGINGIFYYSTSIFQTAGISQPVYATIGVGAINM
gi|2143756|  RQPIVVALMLHVAQQFSGINGIFYYSTSIFQTAGISQPVYATIGVGAINM
gi|6981548|  RQPIVVALMLHVAQQFSGINGIFYYSTSIFQTAGISQPVYATIGVGAINM

                    360       370       380       390       400
             ....|....|....|....|....|....|....|....|....|....|
NOV5         VFTAVSVFLVEKAGRRSLFLIGMSGMFVCAIFMSVGLVLLNKFSWMSYVS
gi|4557851|  VFTAVSVFLVEKAGRRSLFLIGMSGMFVCAIFMSVGLVLLNKFSWMSYVS
gi|17380402| IFTAVSVLLVEKAGRRTLFLTGMIGMFFCTIFMSVGLVLLDKFAWMSYVS
gi|13654262| IFTAVSVLLVEKAGRRTLFLTGMIGMFFCTIFMSVGLVLLDKFAWMSYVS
gi|2143756|  IFTAVSVLLVEKAGRRTLFLAGMIGMFFCAVFMSLGLVLLDKFTWMSYVS
gi|6981548|  IFTAVSVLLVEKAGRRTLFLAGMIGMFFCAVFMSLGLVLLDKFTWMSYVS

                    410       420       430       440       450
             ....|....|....|....|....|....|....|....|....|....|
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```
NOV5            MIAIFLFVSFFEIGPGPIPWFMVAEFFSQGPRPAALAIIAAFSNWTCNFIV
gi|4557851|     MIAIFLFVSFFEIGPGPIPWFMVAEFFSQGPRPAALAIAAFSNWICNFIV
gi|17380402|    MTAIFLFVSFFEIGPGPIPWFMVAEFFSQGPRPTALALAAFSNWVCNFVI
gi|13654262|    MTAIFLFVSFFEIGPGPIPWFMVAEFFSQGPRPTALALAAFSNWVCNFVI
gi|2143756|     MTAIFLFVSFFEIGPGPIPWFMVAEFFSQGPRPTALALAAFSNWVCNFII
gi|6981548|     MTAIFLFVSFFEIGPGPIPWFMVAEFFSQGPRPTALALAAFSNWVCNFII
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                   460       470       480       490       500
                ....|....|....|....|....|....|....|....|....|....|
NOV5            ALCFQYIADFCGPYVFFLFAGVLLAFTLFTFFKVPETKGKSFEEIAAEFQ
gi|4557851|     ALCFQYIADFCGPYVFFLFAGVLLAFTLFTFFKVPETKGKSFEEIAAEFQ
gi|17380402|    ALCFQYIADFLGPYVFFLFAGVVLVFTLFTFFKVPETKGKSFEEIAAEFR
gi|13654262|    ALCFQYIADFLGPYVFFLFAGVVLVFTLFTFFKVPETKGKSFEEIAAEFR
gi|2143756|     ALCFQYIADFLGPYVFFLFAGVVLVFTLFTFFKVPETKGKSFDEIAAEFR
gi|6981548|     ALCFQYIADFLGPYVFFLFAGVVLVFTLFTFFKVPETKGKSFDEIAAEFR
```

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                   510       520
                ....|....|....|....
NOV5            KKSGSAHRPKAAVEMKFLGATETV
gi|4557851|     KKSGSAHRPKAAVEMKFLGATETV
gi|17380402|    KKSGSAPPRKAAVQMEFLASSESV
gi|13654262|    KKSGSAPPRKAAVQMEFLASSESV
gi|2143756|     KKSGSAPPRKATVQMEFLGSSETV
gi|6981548|     KKSGSAPPRKATVQMEFLGSSETV
```

Table 17E lists the domain description from DOMAIN analysis results against NOV5. This indicates that the NOV4 sequence has properties similar to those of other proteins known to contain this domain.

Table 17E Domain Analysis of NOV5
gnl|Pfam|pfam00083, sugar_tr, Sugar (and other) transporter. CD-Length = 447 residues, 99.6% aligned
Score = 344 bits (882), Expect = 8e-96

There are two mechanisms for glucose transport across cell membranes. In the intestine and renal proximal tubule, glucose is transported against a concentration gradient by a secondary active transport mechanism in which glucose is cotransported with sodium ions. In all other cells, glucose transport is mediated by one or more of the members of the closely related GLUT family of glucose transporters. The pattern of expression of the GLUT transporters in different tissues is related to the different roles of glucose metabolism in different tissues. Primary defects in glucose transport all appear to be extremely rare and not all possible deficiencies have been identified. Deficiency of the secondary active sodium/glucose transporters result in glucose/galactose malabsorption or congenital renal glycosuria. GLUT1 deficiency produces a seizure disorder with low glucose concentration in cerebrospinal fluid and GLUT2 deficiency is the basis of the Fanconi-Bickel syndrome, which resembles type I glycogen storage disease. (See Brown, 2000, J Inherit Metab Dis 23(3):237).

The disclosed NOV5 nucleic acid of the invention encoding a glucose transporter type 2 -like protein includes the nucleic acid whose sequence is provided in Table 17A or a

fragment thereof. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 17A while still encoding a protein that maintains its glucose transporter type 2 -like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose

5   sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or

10   derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject. In the mutant or variant nucleic acids, and their complements, up to about 0 percent of the bases may be so changed.

The disclosed NOV5 protein of the invention includes the glucose transporter type 2 -

15   like protein whose sequence is provided in Table 17B. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in Table 17B while still encoding a protein that maintains its glucose transporter type 2- like activities and physiological functions, or a functional fragment thereof. In the mutant or variant protein, up to about 0 percent of the residues may be so changed.

20   The NOV5 nucleic acids and proteins of the invention are useful in potential therapeutic applications implicated in diabetes, fetal growth retardation, cancer, glycogen storage disease, hypertension and/or other disorders and conditions. The NOV5 nucleic acid, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

25   NOV5 nucleic acids and polypeptides are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-NOVX Antibodies" section below. For example the disclosed NOV5 protein have multiple hydrophilic regions,

30   each of which can be used as an immunogen. This novel protein also has value in development of powerful assay system for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders.

NOV6

A disclosed NOV6 nucleic acid of 2184 nucleotides identified as SEQ ID NO:35 (also referred to as CG CG55690-01) encoding a novel Frizzled-9-like protein is shown in Table 18A.

5

Table 18A. NOV6 Nucleotide Sequence (SEQ ID NO:35)
CCGCCTTCGGCCCGGGCCTCCCGGGATGGCCGTGGCGCCTCTGCGGGGGGCGCTGCTGCTGTGGCAGCTGCTGGCGGCGG   GCGGCGCGGCACTGGAGATCGGCCGCTTCGACCCGGAGCGCGGGCGCGGGGCTGCGCCGTGCCAGGCGGTGGAGATCCCC   ATGTGCCGCGGCATCGGCTACAACCTGACCCGCATGCCCAACCTGCTGGGCCACACGTCGCAGGGCGAGGCGGCTGCCGA   GCTAGCGGAGTTCGCGCCGCTGGTGCAGTACGGCTGCCACAGCCACCTGCGCTTCTTCCTGTGCTCGCTCTACGCGCCCA   TGTGCACCGACCAGGTCTCGACGCCCATTCCCGCCTGCCGGCCCATGTGCGAGCAGGCGCGCCTGCGCTGCGCGCCCATC   ATGGAGCAGTTCAACTTCGGCTGGCCGGACTCGCTCGACTGCGCCCGGCTGCCCACGCGCAACGACCCGCACGCGCTGTG   CATGGAGGCGCCCGAGAACGCCACGGCCGGCCCCGCGGAGCCCCACAAGGGCCTGGGCATGCTGCCCGTGGCGCCGCGGC   CCGCGCGCCCTCCCGGAGACCTGGGCCCGGGCGCGGGCGGCAGTGGCACCTGCGAGAACCCCGAGAAGTTCCAGTACGTG   GAGAAGAGCCGCTCGTGCGCACCGCGCTGCGGGCCCGGCGTCGAGGTGTTCTGGTCCCGGCGCGACAAGGACTTCGCGCT   GGTCTGGATGGCCGTGTGGTCGGCGCTGTGCTTCTTCTCCACCGCCTTCACTGTGCTCACCTTCTTGCTGGAGCCCCACC   GCTTCCAGTACCCCGAGCGCCCCATCATCTTCCTCTCCATGTGCTACAACGTCTACTCGCTGGCCTTCCTGATCCGTGCG   GTGGCCGGAGCGCAGAGCGTGGCCTGTGACCAGGAGGCGGGCGCGCTCTACGTGATCCAGGAGGGCCTGGAGAACACGGG   CTGCACGCTGGTCTTCCTACTGCTCTACTACTTCGGCATGGCCAGCTCGCTCTGGTGGGTGGTCCTGACGCTCACCTGGT   TCCTGGCTGCCGGGAAGAAATGGGGCCACGAGGCCATCGAGGCCCACGGCAGCTATTTCCACATGGCTGCCTGGGGCCTG   CCCGCGCTCAAGACCATCGTCATCCTGACCCTGCGCAAGGTGGCGGGTGATGAGCTGACTGGGCTTTGCTACGTGGCCAG   CACGGATGCAGCAGCGCTCACGGGCTTCGTGCTGGTGCCCCTCTCTGGCTACCTGGTGCTGGGCAGTAGTTTCCTCCTGA   CCGGCTTCGTGGCCCTCTTCCACATCCGCAAGATCATGAAGACGGGCGGCACCAACACAGAGAAGCTGGAGAAGCTCATG   GTCAAGATCGGGGTCTTCTCCATCCTCTACACGGTGCCCGCCACCTGCGTCATCGTTTGCTATGTCTACGAACGCCTCAA   CATGGACTTCTGGCGCCTTCGGGCCCACAGAGCAGCCATGCGCAGCGGCCGGCCCGGAGGCCCGGAGGGACTGCTCGC   TGCCAGGGGGCTCGGTGCCCACCGTGGCGGTCTTCATGCTCAAAATTTTCATGTCACTGGTGGTGGGGATCACCAGCGGC   GTCTGGGTGTGGAGCTCCAAGACTTTCCAGACCTGGCAGAGCCTGTGCTACCGCAAGATAGCAGCTGGCCGGGCCCGGGC   CAAGGCCTGCCGCGCCCCCGGGAGCTACGGACGTGGCACGCACTGCCACTATAAGGCTCCCACCGTGGTCTTGCACATGA   CTAAGACGGACCCCTCTTTGGAGAACCCCACACACCTCTAGCCACACAGGCCTGGCGCGGGGTGGCTGCTGCCCCCTCCT   TGCCCTCCACGCCCTGCCCCCTGCATCCCCTAGAGACAGCTGACTAGCAGCTGCCCAGCTGTCAAGGTCAGGCAAGTGAG   CACCGGGGACTGAGGATCAGGGCGGGACCCCGTGAGGCTCATTAGGGGAGATGGGGGTCTCCCCTAATGCGGGGGCTGGA   CCAGGCTGAGTCCCCACAGGGTCCTAGTGGAGGATGTGGAGGGGCGGGGCAGAGGGGTCCAGCCGGAGTTTATTTAATGA   TGTAATTTATTGTTGCGTTCCTCTGGAAGCTGTGACTGGAATAAACCCCCGCGTGGCACTGCTGATCCTCTCTGGCTGGG   AAGGGGGAAGGTAGGAGGTGAGGC

The disclosed NOV6 nucleic acid sequence is located on chromosome 7q11.23.

A disclosed NOV6 polypeptide (SEQ ID NO:36) encoded by SEQ ID NO:35 is 591 amino acid residues and is presented using the one-letter amino acid code in Table 18B.

10   Signal P, Psort and/or Hydropathy results predict that NOV6 contains a signal peptide and is likely to be localized at the plasma membrane with a certainty of 0.6400. In other embodiments, NOV6 is also likely to be localized to the golgi body with a certainty of 0.4600, to the endoplasmic reticulum (membrane) with a certainty of 0.3700, or to the endoplasmic reticulum (lumen) with a certainty of 0.1000. The most likely cleavage site is between

15   positions 22 and 23:GAA-LE.

Table 18B. Encoded NOV6 protein sequence (SEQ ID NO:36).
MAVAPLRGALLLWQLLAAGGAALEIGRFDPERGRGAAPCQAVEIPMCRGIGYNLTRMPNLLGHTSQGEAAAELAEFAPLV   QYGCHSHLRFFLCSLYAPMCTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPHALCMEAPENAT   AGPAEPHKGLGMLPVAPRPARPPGDLGPGAGGSGTCENPEKFQYVEKSRSCAPRCGPGVEVFWSRRDKDFALVWMAVWSA   LCFFSTAFTVLTFLLEPHRFQYPERPIIFLSMCYNVYSLAFLIRAVAGAQSVACDQEAGALYVIQEGLENTGCTLVFLLL   YYFGMASSLWWVVLTLTWFLAAGKKWGHEAIEAHGSYFHMAAWGLPALKTIVILTLRKVAGDELTGLCYVASTDAAALTG   FVLVPLSGYLVLGSSFLLTGFVALFHIRKIMKTGGTNTEKLEKLMVKIGVFSILYTVPATCVIVCYVYERLNMDFWRLRA   TEQPCAAAAGPGGRRDCSLPGGSVPTVAVFMLKIFMSLVVGITSGVWVWSSKTFQTWQSLCYRKIAAGRARAKACRAPGS   YGRGTHCHYKAPTVVLHMTKTDPSLENPTHL

NOV6 is expressed in at least the following tissues: brain, lung and carcinoma tissue. This information was derived by determining the tissue sources of the sequences that were included in the invention including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or RACE sources.

NOV6 has homology to the amino acid sequences shown in the BLASTP data listed in Table 18C.

Table 18C. BLAST results for NOV6					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gi|12861958|dbj|BAB 32311.1|	(AK021164) putative [Mus musculus]	592	95	95	0.0
gi|4689161|gb|AAD27 789.1|AF088850_1 (AF088850)	frizzled-9 [Mus musculus]	592	95	96	0.0
gi|11419362|ref|XP 004646.1| (XM_004646)	frizzled 9 [Homo sapiens]	591	100	100	0.0
gi|5042380|gb|AAB87 503.2| (AF033585)	frizzled-9 protein [Mus musculus]	549	95	96	0.0
gi|9622217|gb|AAF89 677.1|AF169639_1 (AF169639)	Frizzled X [Danio rerio]	577	72	81	0.0

The homology of these sequences is shown graphically in the ClustalW analysis shown in Table 18D.

Table 18D Information for the ClustalW proteins

```
1)   NOV6 (SEQ ID NO:36)
2)   gi|12861958|(SEQ ID NO:126)
3)   gi|17433078|(SEQ ID NO:127)
4)   gi|4503835| (SEQ ID NO:128)
5)   gi|5042380| (SEQ ID NO:129)
6)   gi|9622217| (SEQ ID NO:130)
```

```
                          10        20        30        40        50
                  ....|....|....|....|....|....|....|....|....|....|
NOV6              MAVAP-LRGALLLWQLLAAGGAALEIGRFDPERGRGAAPCQAVEIPMCRG
gi|12861958|      MAVPPLLRGALLLWQLLATGGAALEIGRFDPERGRGPAPCQAMEIPMCRG
gi|17433078|      MAVPPLLRGALLLWQLLATGGAALEIGRFDPERGRGPAPCQAMEIPMCRG
gi|4503835|       MAVAP-LRGALLLWQLLAAGGAALEIGRFDPERGRGAAPCQAVEIPMCRG
gi|5042380|       -----------------------------------------EIPMCRG
gi|9622217|       MGSSP--QIVHSLWCHLVIAAYSLEIGSXDLERGR-PAKCEPTVIPMCSG

                          60        70        80        90        100
                  ....|....|....|....|....|....|....|....|....|....|
NOV6              IGYNLTRMPNLLGHTSQGEAAAELAEFAPLVQYGCHSHLRFFLCSLYAPM
gi|12861958|      IGYNLTRMPNLLGHTSQGEAAAHCAEFSPLVQYGCHSHLRFFRCSLYAPM
gi|17433078|      IGYNLTRMPNLLGHTSQGEAAAQLAEFSPLVQYGCHSHLRFFLCSLYAPM
gi|4503835|       IGYNLTRMPNLLGHTSQGEAAAELAEFAPLVQYGCHSHLRFFLCSLYAPM
gi|5042380|       IGYNLTRMPNLLGHTPQGEAAAQLAEFSPLVQYGCHSHLRFFLCSLYAPM
gi|9622217|       IGYNLTRMPNFMDHDNQREAAIKLNEFAPLVEYGCDVHLRFFLCSLYAPM

                          110       120       130       140       150
                  ....|....|....|....|....|....|....|....|....|....|
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NOV6         CTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPH
gi|12861958| CTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPH
gi|17433078| CTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPH
gi|4503835|  CTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPH
gi|5042380|  CTDQVSTPIPACRPMCEQARLRCAPIMEQFNFGWPDSLDCARLPTRNDPH
gi|9622217|  CTDQVSTSIPACRPMCEQARQECSPIMEKFNXAWPESLNCSKLPTRNDPN

                  160       170       180       190       200
             ....|....|....|....|....|....|....|....|....|....|
NOV6         ALCMEAPENATAGPAEPHKGLGMLPVAPRPARPPGDLGPGAGGSGTCENP
gi|12861958| ALCMEAPENATAGPTEPHKGLGMLPVAPRPARPPGDSAPGPGSGGTCDNP
gi|17433078| ALCMEAPENATAGPTEPHKGLGMLPVAPRPARPPGDSAPGPGSGGTCDNP
gi|4503835|  ALCMEAPENATAGPAEPHKGLGMLPVAPRPARPPGDLGPGAGGSGTCENP
gi|5042380|  ALCMEAPENATAGPTEPHKGLGMLPVAPRPARPPGDSAPGPGSGGTCDNP
gi|9622217|  ALCMEAPENDTK--TETKKGEGMLPVPPPRPRQPGAGNARSGGTMGVCENP

                  210       220       230       240       250
             ....|....|....|....|....|....|....|....|....|....|
NOV6         EKFQYVEKSRSCAPRCGPGVEVFWSRRDKDFALVWMAVWSALCFFSTAFT
gi|12861958| EKFQYVEKSRSCAPRCGPGVEVFWSRRDKDFALVWMAVWSALCFFSTAFT
gi|17433078| EKFQYVEKSRSCAPRCGPGVEVFWSRRDKDFALVWMAVWSALCFFSTAFT
gi|4503835|  EKFQYVEKSRSCAPRCGPGVEVFWSRRDKDFALVWMAVWSALCFFSTAFT
gi|5042380|  EKFQYVEKSRSCAPRCGPGVEVFWSRRDKDFALVWMAVWSALCFFSTAFT
gi|9622217|  EKFQYVEKSETCAPRCSSAVEVFWSRQKDFAFIWMAVWSTLCFVSTAFT

                  260       270       280       290       300
             ....|....|....|....|....|....|....|....|....|....|
NOV6         VLTFLLEPHRFQYPERPIIFLSMCYNVYSLAFLIRAVAGAQSVACDQEAG
gi|12861958| VFTFLLEPHRFQYPERPIIFLSMCYNVYSLAFLIRAVAGAQSVACDQEAG
gi|17433078| VFTFLLEPHRFQYPERPIIFLSMCYNVYSLAFLIRAVAGAQSVACDQEAG
gi|4503835|  VLTFLLEPHRFQYPERPIIFLSMCYNVYSLAFLIRAVAGAQSVACDQEAG
gi|5042380|  VFTFLLEPHRFQYPERPIIFLSMCYNVYSLAFLIRAVAGAQSVACDQEAG
gi|9622217|  VLTFLLDPHRFQYPERPIIFLSMCYNVYSVAFILRSVAGAENTACDRENG

                  310       320       330       340       350
             ....|....|....|....|....|....|....|....|....|....|
NOV6         ALYVIQEGLENTGCTLVFLLLYYFGMASSLWWVVLTLTWFLAAGKKWGHE
gi|12861958| ALYVIQEGLENTGCTLVFLLLYYFGMASSLWWVVLTLTWFLAAGKKWGHE
gi|17433078| ALYVIQEGLENTGCTLVFLLLYYFGMASSLWWVVLTLTWFLAAGKKWGHE
gi|4503835|  ALYVIQEGLENTGCTLVFLLLYYFGMASSLWWVVLTLTWFLAAGKKWGHE
gi|5042380|  ALYVIQEDLENTGCTLVFLLLYYFGMASSLWWVVLTLTWFLAAGKKWGHE
gi|9622217|  ELYIIQEGLESTGCTIVFILYYFGMASSIWWVILTLTWFLAAGKKWGHE

                  360       370       380       390       400
             ....|....|....|....|....|....|....|....|....|....|
NOV6         AIEAHGSYFHMAAWGLPALKTIVILTLRKVAGDELTGLCYVASTDAAALT
gi|12861958| AIEAHGSYFHMAAWGLPALKTIVVLTLRKVAGDELTGLCYVASMDPAALT
gi|17433078| AIEAHGSYFHMAAWGLPALKTIVVLTLRKVAGDELTGLCYVASMDPAALT
gi|4503835|  AIEAHGSYFHMAAWGLPALKTIVILTLRKVAGDELTGLCYVASTDAAALT
gi|5042380|  AIEAHGSYFHMAAWGLPALKTIVVLTLRKVAGDELTGLCYVASMDPAALT
gi|9622217|  AIESHSSYFHMAAWGIPALKTIVILTMRKVAGDELTGLCYVGSMDVGALT

                  410       420       430       440       450
             ....|....|....|....|....|....|....|....|....|....|
NOV6         GFVLVPLSGYLVLGSSFLLTGFVALFHIRKIMKTGGTNTEKLEKLMVKIG
gi|12861958| GFVLVPLSCYLVLGTSFLLTGFVALFHIRKIMKTGGTNTEKLEKLMVKIG
gi|17433078| GFVLVPLSCYLVLGTSFLLTGFVALFHIRKIMKTGGTNTEKLEKLMVKIG
gi|4503835|  GFVLVPLSGYLVLGSSFLLTGFVALFHIRKIMKTGGTNTEKLEKLMVKIG
gi|5042380|  GFVLVPLSCYLVLGTSFLLTGFVALFHIRKIMKTGGTNTEKLEKLMVKIG
gi|9622217|  GFVLVPLSCYLVIGTSFILTGFVALFHIRKVMKTEGTNTEKLEKLMVKIG

                  460       470       480       490       500
             ....|....|....|....|....|....|....|....|....|....|
NOV6         VFSILYTVPATCVIVCYVYERLNMDFWRLRATEQPCAAAAGPGGRR-DCS
gi|12861958| VFSILYTVPATCVIVCYVYERLNMDFWRLRATEQPCTAATVPGGRR-DCS
gi|17433078| VFSILYTVPATCVIVCYVYERLNMDFWRLRATEQPCTAATVPGGRR-DCS
gi|4503835|  VFSILYTVPATCVIVCYVYERLNMDFWRLRATEQPCAAAAGPGGRR-DCS
gi|5042380|  VFSILYTVPATCVIVCYVYERLNMDFWRLRATEQPCTAATVPGGRR-DCS
gi|9622217|  IYSILYTVPATCVIICYFYERLNMDYWKFRGLQSKCT--TFPGRRNEDCS

                  510       520       530       540       550
             ....|....|....|....|....|....|....|....|....|....|
NOV6         LPGGSVPTVAVFMLKIFMSLVVGITSGVWVWSSKTFQTWQSLCYRKIAAG
gi|12861958| LPGGSVPTVAVFMLKIFMSLVVGITSGVWVWSSKTFQTWQSLCYRKMAAG
gi|17433078| LPGGSVPTVAVFMLKIFMSLVVGITSGVWVWSSKTFQTWQSLCYRKIAAG
gi|4503835|  LPGGSVPTVAVFMLKIFMSLVVGITSGVWVWSSKTFQTWQSLCYRKIAAG
gi|5042380|  LPGGSVPTVAVFMLKIFMSLVVGITSGVWVWSSKTFQTWQSLCYRKMAAG
gi|9622217|  LDS-SVPTVAVFILKIFMSLVVGITSGVWVWSSKTLQTWQGLCSRKITD-
```

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              560       570       580       590
         ....|....|....|....|....|....|....|....|...
NOV6         RARAKACRAPGSYGRGTHCHYKAPTVVLHMTKTDPSLENPTHL
gi|12861958| RARAKACRTPGGYGRGTHCHYKAPTVVLHMTKTDPSLENPTHP
gi|17433078| RARAKACRTPGGYGRGTHCHYKAPTVVLHMTKTDPSLENPTHL
gi|4503835|  RARAKACRAPGSYGRGTHCHYKAPTVVLHMTKTDPSLENPTHL
gi|5042380|  RARAKACRTPGGYGRGTHCHYKAPTVVLHMTKTDPSLENPTHL
gi|9622217|  RTCRKHCSTS-------HCHYKAPAVELHMSKTDPYSDCPTHV
```

Tables 18E list the domain description from DOMAIN analysis results against NOV6. This indicates that the NOV6 sequence has properties similar to those of other proteins known to contain this domain.

Table 18E. Domain Analysis of NOV6
gnl|Pfam|pfam01534, Frizzled, Frizzled CD-Length = 328 residues, 98.5% aligned Score = 440 bits (1131), Expect = 1e-124

Epithelial cell differentiation and morphogenesis are crucial in many aspects of metazoan development. Recent genetic studies in Drosophila have revealed that the conserved Jun amino-terminal kinase (JNK) signaling pathway regulates epithelial morphogenesis during the process of embryonic dorsal closure and participates in the control of planar polarity in several tissues. Importantly, these studies have linked the JNK pathway to the decapentaplegic and Frizzled pathways in these processes, suggesting a high degree of integrative signaling during epithelial morphogenesis. (See Noselli et al., 1999, Curr Opin Genet Dev 9:466-72).

The wnt signaling pathway has important functions in nervous system development. To better understand this process we have cloned and analyzed the expression of the wnt receptor, frizzled 9, in the developing nervous system in mouse, chick and zebrafish. The earliest expression of mouse frizzled 9 mRNA expression begins at E8.5 with expression throughout the entire rostral-caudal neuraxis. This early expression pattern within the neural tube appears to be conserved between chick and zebrafish. Expression becomes restricted to a ventral domain in the mouse ventricular zone at E11.5, a region specified to give rise to neurons and glia. Using a polyclonal antibody to MFZ9 further shows expression limited to neural restricted precursors cells. (See Van Ray et al., 2001, Dev Genes Evol, 211(8-9):453-7).

The disclosed NOV6 nucleic acid of the invention encoding a Frizzled-9-like protein includes the nucleic acid whose sequence is provided in Table 18A or a fragment thereof. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 18A while still encoding a protein that maintains its Frizzled-9-like activities and physiological functions, or a fragment of such a nucleic acid.

The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications

5   include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject. In the mutant or variant nucleic acids, and their complements, up to about 5 percent of the

10 bases may be so changed.

The disclosed NOV6 protein of the invention includes the Frizzled-9-like protein whose sequence is provided in Table 18B. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in Table 18B while still encoding a protein that maintains its Frizzled-9-like activities and

15 physiological functions, or a functional fragment thereof. In the mutant or variant protein, up to about 5 percent of the residues may be so changed.

The above defined information for this invention suggests that these Frizzled-9-like proteins (NOV6) may function as a member of a "Frizzled-9 family". Therefore, the NOV6 nucleic acids and proteins identified here may be useful in potential therapeutic applications

20 implicated in (but not limited to) various pathologies and disorders as indicated below. The potential therapeutic applications for this invention include, but are not limited to: protein therapeutic, small molecule drug target, antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), diagnostic and/or prognostic marker, gene therapy (gene delivery/gene ablation), research tools, tissue regeneration *in vivo* and *in vitro* of all tissues

25 and cell types composing (but not limited to) those defined here.

The nucleic acids and proteins of NOV6 are useful in ulcerative colitis, Crohn's disease, recessive Robinow syndrome, cancer and/or other pathologies/disorders.

NOV6 nucleic acids and polypeptides are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or

30 diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-NOVX Antibodies" section below. For example the disclosed NOV6 protein have multiple hydrophilic regions, each of which can be used as an immunogen. This novel protein also has value in development of powerful assay system for functional analysis of various human disorders,

which will help in understanding of pathology of the disease and development of new drug targets for various disorders.

NOV7

5   A disclosed NOV nucleic acid of 2523 nucleotides (also referred to CG55117-01) encoding a novel prominin-like protein is shown in Table 19A.

Table 19A. NOV7 Nucleotide Sequence (SEQ ID NO:37)
GGATCCGGAGGGCAGCCTTCATCCACAGATGCTCCTAAGGCTTGGAATTATGAATTGCCT GCAACAAATTATGAGACCCAAGACTCCCATAAAGCTGGACCCATTGGCATTCTCTTTGAA CTAGTGCATATCTTTCTCTATGTGGTACAGCCGCGTGATTTCCCAGAAGATACTTTGAGA AAATTCTTACAGAAGGCATATGAATCCAAAATTGATTATGACAAGATTGTCTACTATGAA GCAGGGATTATTCTATGCTGTGTCCTGGGGCTGCTGTTTATTATTCTGATGCCTCTGGTG GGGTATTTCTTTTGTATGTGTCGTTGCTGTAACAAATGTGGTGGAGAAATGCACCAGCGA CAGAAGGAAAATGGGCCCTTCCTGAGGAAATGCTTTGCAATCTCCCTGTTGGTGATTTGT ATAATAATAAGCATTGGCATCTTCTATGGTTTTGTGGCAAATCACCAGGTAAGAACCCGG ATCAAAAGGAGTCGGAAACTGGCAGATAGCAATTTCAAGGACTTGCGAACTCTCTTGAAT GAAACTCCAGAGCAAATCAAATATATATTGGCCCAGTACAACACTACCAAGGACAAGGCG TTCACAGATCTGAACAGTATCAATTCAGTGCTAGGAGGCGGAATTCTTGACCGACTGAGA CCCAACATCATCCCTGTTCTTGATGAGATTAAGTCCATGGCAACAGCGATCAAGGAGACC AAAGAGGCGTTGGAGAACATGAACAGCACCTTGAAGAGCTTGCACCAACAAAGTACACAG CTTAGCAGCAGTCTGACCAGCGTGAAAACTAGCCTGCGGTCATCTCTCAATGACCCTCTG TGCTTGGTGCATCCATCAAGTGAAACCTGCAACAGCATCAGATTGTCTCTAAGCCAGCTG AATAGCAACCCTGAACTGAGGCAGCTTCCACCCGTGGATGCAGAACTTGACAACGTTAAT AACGTTCTTAGGACAGATTTGGATGGCCTGGTCCAACAGGGCTATCAATCCCTTAATGAT ATACCTGACAGAGTACAACGCCAAACCACGACTGTCGTAGCAGGTATCAAAAGGGTCTTG AATTCCATTGGTTCAGATATCGACAATGTAACTCAGCGTCTTCCTATTCAGGATATACTC TCAGCATTCTCTGTTTATGTTAATAACACTGAAAGTTACATCCACAGAAATTTACCTACA TTGGAAGAGTATGATTCATACTGGTGGCTGGGTGGCCTGGTCATCTGCTCTCTGCTGACC CTCATCGTGATTTTTTACTACCTGGGCTTACTGTGTGGCGTGTGCGGCTATGACAGGCAT GCCACCCCGACCACCCGAGGCTGTGTCTCCAACACCGGAGGCGTCTTCCTCATGGTTGGA GTTGGATTAAGTTTCCTCTTTTGCTGGATATTGATGATCATTGTGGTTCTTACCTTTGTC TTTGGTGCAAATGTGGAAAAACTGATCTGTGAACCTTACACGAGCAAGGAATTATTCCGG GTTTTGGATACACCCTACTTACTAAATGAAGACTGGGAATACTATCTCTCTGGGAAGCTA TTTAATAAATCAAAAATGAAGCTCACTTTTGAACAAGTTTACAGTGACTGCAAAAAAAAT AGAGGCACTTACGGCACTCTTCACCTGCAGAACAGCTTCAATATCAGTGAACATCTCAAC ATTAATGAGCATACTGGAAGCATAAGCAGTGAATTGGAAAGTCTGAAGGTAAATCTTAAT ATCTTTCTGTTGGGTGCAGCAGGAAGAAAAAACCTTCAGGATTTTGCTGCTTGTGGAATA GACAGAATGGATTATGACAGCTACTTGGCTCAGACTGGTAAATCCCCCGCAGGAGTGAAT CTTTTATCATTTGCATATGATCTAGAAGCAAAAGCAAACAGTTTGCCCCCAGGAAATTTG AGGAACTCCCTGAAAAGAGATGCACAAACTATTAAAACAATTCACCAGCAACGAGTCCTT CCTATAGAACAATCACTGAGCACTCTATACCAAAGCGTCAAGATACTTCAACGCACAGGG AATGGATTGTTGGAGAGAGTAACTAGGACTCTAGCTTCTCTGGATTTTGCTCAGAACTTC ATCACAAACAATACTTCCTCTGTTATTATTGAGGAAACTAAGAAGTATGGGAGGACAATA ATAGGATATTTTGAACATTATCTGCAGTGGATCGAGTTCTCTATCAGTGAGAAAGTGGCA TCGTGCAAACCTGTGGCCACCGCTCTAGATACTGCTGTTGATGTCTTTCTGTGTAGCTAC ATTATCGACCCCTTGAATTTGTTTTGGTTTGGCATAGGAAAAGCTACTGTATTTTTACTT CCGGCTCTAATTTTTGCGGTAAAACTGGCTAAGTACTATCGTCGAATGGATTCGGAGGAC GTGTACGATGATGTTGAAACTATACCCATGAAAAATATGGAAAATGGTAATAATGGTTAT CATAAAGATCATGTATATGGTATTCACAATCCTGTTATGACAAGCCCATCACAACATCTCGAG

A disclosed NOV7 polypeptide (SEQ ID NO:38) encoded by SEQ ID NO:37 is 837 amino acid residues and is presented using the one-letter amino acid code in Table 19B.

Signal P, Psort and/or Hydropathy results predict that NOV7 has no signal peptide and is

likely to be localized in the plasma membrane with a certainty of 0.6400. In other

embodiments, NOV7 is also likely to be localized to the Golgi body with a certainty of 0.4000,

in the endoplasmic reticulum (membrane) with a certainty of 0.300, or to the mitochondrial

5   inner membrane with a certainty of 0.1000.

Table 19B. Encoded NOV7 protein sequence (SEQ ID NO:38).
GGQPSSTDAPKAWNYELPATNYETQDSHKAGPIGILFELVHIFLYVVQPRDFPEDTLRKF LQKAYESKIDYDKIVYYEAGIILCCVLGLLFIILMPLVGYFFCMCRCCNKCGGEMHQRQK ENGPFLRKCFAISLLVICIIISIGIFYGFVANHQVRTRIKRSRKLADSNFKDLRTLLNET PEQIKYILAQYNTTKDKAFTDLNSINSVLGGGILDRLRPNIIPVLDEIKSMATAIKETKE ALENMNSTLKSLHQQSTQLSSSLTSVKTSLRSSLNDPLCLVHPSSETCNSIRLSLSQLNS NPELRQLPPVDAELDNVNNVLRTDLDGLVQQGYQSLNDIPDRVQRQTTTVVAGIKRVLNS IGSDIDNVTQRLPIQDILSAFSVYVNNTESYIHRNLPTLEEYDSYWWLGGLVICSLLTLI VIFYYLGLLCGVCGYDRHATPTTRGCVSNTGGVFLMVGVGLSFLFCWILMIIVVLTFVFG ANVEKLICEPYTSKELFRVLDTPYLLNEDWEYYLSGKLFNKSKMKLTFEQVYSDCKKNRG TYGTLHLQNSFNISEHLNINEHTGSISSELESLKVNLNIFLLGAAGRKNLQDFAACGIDR MDYDSYLAQTGKSPAGVNLLSFAYDLEAKANSLPPGNLRNSLKRDAQTIKTIHQQRVLPI EQSLSTLYQSVKILQRTGNGLLERVTRTLASLDFAQNFITNNTSSVIIEETKKYGRTIIG YFEHYLQWIEFSISEKVASCKPVATALDTAVDVFLCSYIIDPLNLFWFGIGKATVFLLPA LIFAVKLAKYYRRMDSEDVYDDVETIPMKNMENGNNGYHKDHVYGIHNPVMTSPSQH

NOV7 is expressed in at least the following tissues: ovary. This information was

derived by determining the tissue sources of the sequences that were included in the invention

10   including but not limited to SeqCalling sources, Public EST sources, Literature sources, and/or

RACE sources.

NOV7 has homology to the amino acid sequence shown in the BLASTP data listed in

Table 19C.

Table 19C. BLAST results for NOV7					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
gb|AAH12089.1|AAH120 89 (BC012089)	Similar to prominin (mouse)- like 1 [Homo sapiens]	856	99	99	0.0
sp|O43490|PML1_HUMAN	Human PROMININ- LIKE PROTEIN 1 PRECURSOR (ANTIGEN AC133)	865	99	99	0.0
gb|AAK82364.1|AF3867 58_1 (AF386758)	prominin [Rattus norvegicus]	857	61	79	0.0
gb|AAB96916.1| (AF039663)	AC133 antigen homolog [Mus musculus]	867	60	78	0.0
ref|NP_032961.1| (NM_008935)	prominin [Mus musculus]	858	60	78	0.0

15

The homology of these sequences is shown graphically in the ClustalW analysis shown in Table 19D.

Table 7D. Information for the ClustalW proteins

```
1)  NOV7   (SEQ ID NO: 38)
2)  gb|AAH12089.1|   (SEQ ID NO:131)
3)  ref|NP_006008.1| (SEQ ID NO:132)
4)  gb|AAK82364.1|   (SEQ ID NO:133)
5)  sp|O54990|       (SEQ ID NO:134)
6)  ref|NP_032961.1| (SEQ ID NO:135)
```

```
                        10        20        30        40        50
              ....|....|....|....|....|....|....|....|....|....|....|
NOV7          ----------------------------------------------------
gb|AAH12089.1|    MALVLGSLLLLGLCGNSFSGGQPSSTDAPKAWNYELPAINYETQDSHKAG
ref|NP_006008.1|  MALVLGSLLLLGLCGNSFSGGQPSSTDAPKAWNYELPAINYETQDSHKAG
gb|AAK82364.1|    MALVFSVLLLLGLCGKMASGGQPAFDNTPGALNYELPTTEYETQDIFNAG
sp|O54990|        MALVFSALLLLGLCGKISSEGQPAFHNTPGAMNYELPTTKYETQDIFNAG
ref|NP_032961.1|  MALVFSALLLLGLCGKISSEGQPAFHNTPGAMNYELPTTKYETQDIFNAG
```

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                        60        70        80        90        100
              ....|....|....|....|....|....|....|....|....|....|
NOV7          ----------------------------------------------------
gb|AAH12089.1|    PIGILPELVHIFLYVVQPRDFPEDTLRKFLQKA-YESKIDYDK-------
ref|NP_006008.1|  PIGILPELVHIFLYVVQPRDFPEDTLRKFLQKA-YESKIDYDKPETVILG
gb|AAK82364.1|    ILDPLYQMVHIFLNVVQPNDFPQDLVKKLIQKR-EDISVDTKE-------
sp|O54990|        IVGPLYKMVHIFLSVVQPNDFPIDLIKKLIQNKKEDISVDSKEPEIIVLA
ref|NP_032961.1|  IVGPLYKMVHIFLNVVQPNDFPIDLIKKLIQNKNEDISVDSKE-------
```

```
                        110       120       130       140       150
              ....|....|....|....|....|....|....|....|....|....|
NOV7          -----------------------------------------------E
gb|AAH12089.1|    --IVYYEAGEIVCCVLGLLFIILMPLVGYFFCMCRCCNKCGGEMHQRQKE
ref|NP_006008.1|  LKIVYYEAGEIVCCVLGLLFIILMPLVGYFFCMCRCCNKCGGEMHQRQKE
gb|AAK82364.1|    --VAIYEIGVLICVLLGLLFIFLMPLVGFFFCMCRCCNKCGGEMHQRQKQ
sp|O54990|        LKIALYEIGVLICALLGLLFIILMPLVGCFFCMCRCCNKCGGEMHQRQKQ
ref|NP_032961.1|  --IALYEIGVLICALLGLLFIILMPLVGCFFCMCRCCNKCGGEMHQRQKQ
```

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                        160       170       180       190       200
              ....|....|....|....|....|....|....|....|....|....|
NOV7          NGPFLRKCFAISLLVICLTISIGIFYGFVANHQVRTRIKRSRKLADSNFK
gb|AAH12089.1|    NGPFLRKCFAISLLVICLTISIGIFYGFVANHQVRTRIKRSRKLADSNFK
ref|NP_006008.1|  NGPFLRKCFAISLLVICLTISIGIFYGFVANHQVRTRIKRSRKLADSNFK
gb|AAK82364.1|    NESCRRKCLAISLLLICLLMSLGIAEGFVANQQTRTRIQRRQKLAESNFR
sp|O54990|        NAPCRRKCLGLSLLVICLLMSIGIIYGFVANQQTRTRIKGTQKLAKSNFR
ref|NP_032961.1|  NAPCRRKCLGLSLLVICLLMSLGIIYGFVANQQTRTRIKGTQKLAKSNFR
```

```
                        210       220       230       240       250
              ....|....|....|....|....|....|....|....|....|....|
NOV7          DLRTLLNETPEQIKYILAQYNTTKDKAFIDLNSINSVLGGGILDRLRPNI
gb|AAH12089.1|    DLRTLLNETPEQIKYILAQYNTTKDKAFIDLNSINSVLGGGILDRLRPNI
ref|NP_006008.1|  DLRTLLNETPEQIKYILAQYNTTKDKAFIDLNSINSVLGGGILDRLRPNI
gb|AAK82364.1|    DLRALLTEAPKQIDYILGQYNTTKNKAFSDLDSIDSVLGGRIKGQLKPKV
sp|O54990|        DFQTLLTETPKQIDYVVEQYTNTKNKAFSDLDGIGSVLGGRIKDQLKPKV
ref|NP_032961.1|  DFQTLLTETPKQIDYVVEQYTNTKNKAFSDLDGIGSVLGGRIKDQLKPKV
```

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                        260       270       280       290       300
              ....|....|....|....|....|....|....|....|....|....|
NOV7          IPVLDEIKSMATAIKETKEALENMNSILKSLHQQSTQLSSSLTSVKTSLR
gb|AAH12089.1|    IPVLDEIKSMATAIKETKEALENMNSILKSLHQQSTQLSSSLTSVKTSLR
ref|NP_006008.1|  IPVLDEIKSMATAIKETKEALENMNSILKSLHQQSTQLSSSLTSVKTSLR
gb|AAK82364.1|    TPVLEEIKAMATAIRQTKDALQNMSSSLKSLRDASTQLSINLTSVRNSIE
sp|O54990|        TPVLEEIKAMATAIKQTKDALQNMSSSLKSLQDAATQLNINLSSVRNSIE
ref|NP_032961.1|  TPVLEEIKAMATAIKQTKDALQNMSSSLKSLQDAATQLNINLSSVRNSIE
```

```
                        310       320       330       340       350
              ....|....|....|....|....|....|....|....|....|....|
NOV7          SSLNDPLCLVHPSSETCNSIRLSLSQLNSNPELRQLPPVDAELDNVNNVL
gb|AAH12089.1|    SSLNDPLCLVHPSSETCNSIRLSLSQLNSNPELRQLPPVDAELDNVNNVL
ref|NP_006008.1|  SSLNDPLCLVHPSSETCNSIRLSLSQLNSNPELRQLPPVDAELDNVNNVL
gb|AAK82364.1|    NSLNSNDCASDPASKICDSLRPQLSNLGSNHNGSQLPSVDRELNTVNDVD
sp|O54990|        NSLSSSDCTSDPASKICDSIRPGLSSLGSSLNSSQLPSVDRELNTVTEVD
ref|NP_032961.1|  NSLSSSDCTSDPASKICDSIRPGLSSLGSSLNSSQLPSVDRELNTVTEVD
```

```
                                 360       370       380       390       400
                          ....|....|....|....|....|....|....|....|....|....|
        NOV7              RTDLDGLVQQGYQSLNDIPDRVQRQTTTVMAGIKRVLNSIGSDIDNVIQR
        gb|AAH12089.1|    RTDLDGLVQQGYQSLNDIPDRVQRQTTTVMAGIKRVLNSIGSDIDNVIQR
        ref|NP_006008.1|  RTDLDGLVQQGYQSLNDIPDRVQRQTTTVMAGIKRVLNSIGSDIDNVIQR
        gb|AAK82364.1|    RTDLESLVKRGYMSIDEIPNMTQNQTGDVIKDVKKTLDSVSSKVKNMSQS
        sp|O54990|        KTDLESLVKRGYTIDEIPNTIQNQTVDVIKDVKNTLDSISSNIKDMSQS
        ref|NP_032961.1|  KTDLESLVKRGYTIDEIPNTIQNQTVDVIKDVKNTLDSISSNIKDMSQS

                                 410       420       430       440       450
                          ....|....|....|....|....|....|....|....|....|....|
        NOV7              LPIQDILSAFSVYMNNTESYIHRNLPTLEEYDSYWWLGGLVICSLLTLIV
        gb|AAH12089.1|    LPIQDILSAFSVYMNNTESYIHRNLPTLEEYDSYWWLGGLVICSLLTLIV
        ref|NP_006008.1|  LPIQDILSAFSVYMNNTESYIHRNLPTLEEYDSYWWLGGLVICSLLTLIV
        gb|AAK82364.1|    IPVEEVLLQFSHYLNDSNRYIHESLPRVEEYDSYWWLGGLIVCFLLTLIV
        sp|O54990|        IPIEDMLLQVSHYLNNSNRYLNQELPKLEEYDSYWWLGGLIVCFLLTLIV
        ref|NP_032961.1|  IPIEDMLLQVSHYLNNSNRYLNQELPKLEEYDSYWWLGGLIVCFLLTLIV

                                 460       470       480       490       500
                          ....|....|....|....|....|....|....|....|....|....|
        NOV7              IFYYLGLLCGVCGYDRHATPTTRGCVSNTGGVFLMVGVGLSFLFCWILMI
        gb|AAH12089.1|    IFYYLGLLCGVCGYDRHATPTTRGCVSNTGGVFLMVGVGLSFLFCWILMI
        ref|NP_006008.1|  IFYYLGLLCGVCGYDRHATPTTRGCVSNTGGVFLMVGVGLSFLFCWILMI
        gb|AAK82364.1|    TFYYLGLLCGVFGYDKRATPTRRGCVSNTGGIFLMAGVGFSFLFCWILMI
        sp|O54990|        TFFYLGLLCGVFGYDKHATPTRRGCVSNTGGIFLMAGVGFGFLFCWILMI
        ref|NP_032961.1|  TFFYLGLLCGVFGYDKHATPTRRGCVSNTGGIFLMAGVGFGFLFCWILMI

                                 510       520       530       540       550
                          ....|....|....|....|....|....|....|....|....|....|
        NOV7              IVVLTFVFGANVEKLICEPYTSKELFRVLDTPYLLNEDWDYYLSGKLFNK
        gb|AAH12089.1|    IVVLTFVFGANVEKLICEPYTSKELFRVLDTPYLLNEDWDYYLSGKLFNK
        ref|NP_006008.1|  IVVLTFVFGANVEKLICEPYTSKELFRVLDTPYLLNEDWDYYLSGKLFNK
        gb|AAK82364.1|    LVVLTFVVGANVEKLLCEPYENKKLLQVLDTPYLLNDQWQYYLSGILLKN
        sp|O54990|        VVVLTFVVGANVEKLLCEPYENKKLLQVLDTPYLLKEQWQYYLSGMLFNN
        ref|NP_032961.1|  LVVLTFVVGANVEKLLCEPYENKKLLQVLDTPYLLKEQWQYYLSGMLFNN

                                 560       570       580       590       600
                          ....|....|....|....|....|....|....|....|....|....|
        NOV7              SKMKLTFEQVYSDCKKNRGTYGTLHLQNSFNISEHLNINEHTGSISSELE
        gb|AAH12089.1|    SKMKLTFEQVYSDCKKNRGTYGTLHLQNSFNISEHLNINEHTGSISSELE
        ref|NP_006008.1|  SKMKLTFEQVYSDCKKNRGTYGTLHLQNSFNISEHLNINEHTGSISSELE
        gb|AAK82364.1|    PDINMTFEQVYRDCKRGRGVYAIFQLENVFNITENFNIERLSEDIVKELE
        sp|O54990|        PDINMTFEQVYRDCKRGRGIYAAFQLENVVNVSDHFNIDQISENINTELE
        ref|NP_032961.1|  PDINMTFEQVYRDCKRGRGIYAAFQLENVVNVSDHFNIDQISENINTELE

                                 610       620       630       640       650
                          ....|....|....|....|....|....|....|....|....|....|
        NOV7              SLKVNLN-IFLLGAAGRKNLQDFAACGIDRMDYDSYLAQTGKSPAGVNLL
        gb|AAH12089.1|    SLKVNLN-IFLLGAAGRKNLQDFAACGIDRMNYDSYLAQTGKSPAGVNLL
        ref|NP_006008.1|  SLKVNLN-IFLLGAAGRKNLQDFAACGIDRMNYDSYLAQTGKSPAGVNLL
        gb|AAK82364.1|    KLNVNIDSIELLDKTGRKSLEDFAQSGIDRINYSMYLQEAEKPPTKVDLL
        sp|O54990|        NLNVNIDSIELLDNTGRKSLEDFAHSGIDTIDYSTYLKETEKSPTEVNLL
        ref|NP_032961.1|  NLNVNIDSIELLDNTGRKSLEDFAHSGIDTIDYSTYLKETEKSPTEVNLL

                                 660       670       680       690       700
                          ....|....|....|....|....|....|....|....|....|....|
        NOV7              SFAYDLEAKANSLPPGNLRNSLKRDAQTIKTIHQQRVLPEQSLSTLYQS
        gb|AAH12089.1|    SFAYDLEAKANSLPPGNLRNSLKRDAQTIKTIHQQRVLPEQSLSTLYQS
        ref|NP_006008.1|  SFAYDLEAKANSLPPGNLRNSLKRDAQTIKTIHQQRVLPEQSLSTLYQS
        gb|AAK82364.1|    TFASFLETEANQLPDGNLKQAFLMDAQNIRAIHQQHVPPVGQSLNSLKQS
        sp|O54990|        IFASTLEAKANQLPEGKPKQAFLLDVQNIRAIHQHLPPVGQSLNTLRQS
        ref|NP_032961.1|  IFASTLEAKANQLPEGKPKQAFLLDVQNIRAIHQHLPPVGQSLNTLRQS

                                 710       720       730       740       750
                          ....|....|....|....|....|....|....|....|....|....|
        NOV7              VKILQRTGNGLLERVTRTLASLDFAQNFITNNTSSVIIEETKKYGRTIIG
        gb|AAH12089.1|    VKILQRTGNGLLERVTRILASLDFAQNFITNNTSSVIIEETKKYGRTIIG
        ref|NP_006008.1|  VKILQRTGNGLLERVTRILASLDFAQNFITNNTSSVIIEETKKYGRTIIG
        gb|AAK82364.1|    VWALKQTSSKLPEEVKKVLASLDSAQHFLTSNLSSIVICETKKFGRTIIG
        sp|O54990|        VWTLQQTSNKLPEKVKKILASLDSVQHFLTNNVSLIVICETKKFGKTIIG
        ref|NP_032961.1|  VWTLQQTSNKLPEKVKKILASLDSVQHFLTNNVSLIVICETKKFGKTIIG

                                 760       770       780       790       800
                          ....|....|....|....|....|....|....|....|....|....|
        NOV7              YFEHYLQWIEFSISEKVASCKPVATALDTAVDVFLCSYIIDPLNLFWFGI
        gb|AAH12089.1|    YFEHYLQWIEFSISEKVASCKPVATALDTAVDVFLCSYIIDPLNLFWFGI
        ref|NP_006008.1|  YFEHYLQWIEFSISEKVASCKPVATALDTAVDVFLCSYIIDPLNLFWFGI
        gb|AAK82364.1|    YFEHYLQWMLVAITEKMTSCKPMITAMDSAVNGILCSYVADPLNLFWFGI
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sp|O54990|      YFEHYLHWMFXAIDEKMTSCKPMATAMDSAVNGILCGYVADPLNLFWFGI
ref|NP_032961.1| YFEHYLHWVFXAIDEKMTSCKPMATAMDSAVNGILCGYVADPLNLFWFGI

                      810       820       830       840       850
                 ....|....|....|....|....|....|....|....|....|....|
NOV7             GKATVFLLPADIPAMKLAKYYRRMDSEDVYDDVETIPMKNMENGNNGYHK
gb|AAH12089.1|   GKATVFLLPADIPAMKLAKYYRRMDSEDVYDDVETIPMKNMENGNNGYHK
ref|NP_006008.1| GKATVFLLPADIPAMKLAKYYRRMDSEDVYDDVETIPMKNMENGNNGYHK
gb|AAK82364.1|   GKATMLLLPAVIIAIKLAKYYRRMDSEDVYDDVETIPMKNLENGSNGYHK
sp|O54990|       GKATVLLLPAVIIAIKLAKYYRRMDSEDVYDDVETVPMKNLEIGSNGYHK
ref|NP_032961.1| GKATVLLLPAVIIAIKLAKYYRRMDSEDVYDDVETVPMKNLEIDSNGYHK

                      860
                 ....|....|....|..
NOV7             DHMYGIHNPVMTSPSQH
gb|AAH12089.1|   DHMYGIHNPVMTSPSQH
ref|NP_006008.1| DHMYGIHNPVMTSPSQH
gb|AAK82364.1|   DHLYGMHNPVMTSPSRY
sp|O54990|       DHLYGMHNPVMTSPSRY
ref|NP_032961.1| DHLYGMHNPVMTSPSRY
```

Prominin is the first identified member of a novel family of polytopic membrane proteins conserved throughout the animal kingdom. It has an unusual membrane topology, containing five transmembrane domains and two large glycosylated extracellular loops. In mammals, prominin is expressed in various embryonic and adult epithelial cells, as well as in nonepithelial cells, such as hematopoietic stem cells. At the subcellular level, prominin is selectively localized in microvilli and other plasma membrane protrusions, irrespective of cell type. At the molecular level, prominin specifically interacts with membrane cholesterol and is a marker of a novel type of cholesterol-based lipid 'raft'. A frameshift mutation in the human prominin gene, which results in a truncated protein that is no longer transported to the cell surface, is associated with retinal degeneration. Given that prominin is concentrated in the plasma membrane evaginations at the base of the outer segment of rod photoreceptor cells, which are essential precursor structures in the biogenesis of photoreceptive disks, it is proposed that prominin has a role in the generation of plasma membrane protrusions, their lipid composition and organization and their membrane-to-membrane interactions. (See Corbeil et al., 2001, Traffic 2(2):82-91).

The disclosed NOV7 nucleic acid of the invention encoding a prominin-like protein includes the nucleic acid whose sequence is provided in Table 19A or a fragment thereof. The invention also includes a mutant or variant nucleic acid any of whose bases may be changed from the corresponding base shown in Table 19A while still encoding a protein that maintains its prominin-like activities and physiological functions, or a fragment of such a nucleic acid. The invention further includes nucleic acids whose sequences are complementary to those just described, including nucleic acid fragments that are complementary to any of the nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications. Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar

phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject. In the mutant or variant nucleic acids, and their complements, up to about 5 percent of the bases may be so changed.

The disclosed NOV7 protein of the invention includes the prominin-like protein whose sequence is provided in Table 19B. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in Table 7B while still encoding a protein that maintains its prominin-like activities and physiological functions, or a functional fragment thereof. In the mutant or variant protein, up to about 5 percent of the residues may be so changed.

The protein similarity information, expression pattern, and map location for the prominin-like protein and nucleic acid (NOV7) disclosed herein suggest that NOV7 may have important structural and/or physiological functions characteristic of the prominin-like family. Therefore, the NOV7 nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed, as well as potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), and (v) a composition promoting tissue regeneration in vitro and in vivo.

The NOV7 nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications implicated in various diseases and disorders described below and/or other pathologies. For example, the compositions of the present invention will have efficacy for treatment of patients suffering from neurological disorders, cholesterol transport disorders, retinal degeneration and/or other pathologies/disorders. The NOV7 nucleic acid, or fragments thereof, may further be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed.

NOV7 nucleic acids and polypeptides are further useful in the generation of antibodies that bind immunospecifically to the novel substances of the invention for use in therapeutic or diagnostic methods. These antibodies may be generated according to methods known in the art, using prediction from hydrophobicity charts, as described in the "Anti-NOVX Antibodies" section below. For example the disclosed NOV7 protein have multiple hydrophilic regions,

each of which can be used as an immunogen. This novel protein also has value in development of powerful assay system for functional analysis of various human disorders, which will help in understanding of pathology of the disease and development of new drug targets for various disorders.

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NOV8

A disclosed NOV8 nucleic acid of 2363 nucleotides identified as SEQ ID NO:39 (also referred to as CG55606) encoding a novel hepsin-like protein is shown in Table 20A.

Table 20A. NOV8 nucleotide sequence (SEQ ID NO:39).
AATCAAAACCATCTTTATTATTTAAAGAGCATCCCATCATCAGGGGCACCTAGACAGGAGTCCCAGACAG
CAGAACAATATTTACATGGGGGTCAGGAGGGTGAGGTTGGGTGGTCTCGGGGCTGAGTGGGCCCGCCACT
GTGGAAGAGAGGACCCTGGAGGGAGGGTGTCCTTGGACCTGTGGACCGGGCCCAAGAAGAAAAACGTCCC
ATCCTCGGCCCAGCGTGGATCCCACCACCGGGATCACCTCGGGCCCTGGAGGCTGCGCAGCGAGAAGCCA
CCGGTCAGAGCTGGGTCACCATGCCGCTGGCTTCGGAGTGAGTCTTTATGGCCTGGAAGATCCACTCCCG
GAAGTCACTGACTTTGGTGTAGACGCCTGGCTTCTGGGCCAGGGCACAGCCAGTGCCCCAACTCACAATG
CCACACAGCCGCCAACGTGGCGTCCGAGAGATGCTGTCCTCACACACAAAGGGACCACCGCTGTCGCCCT
GGCAGGCATCAATGCCACCCTCGGGGTAGCCAGCACAGAACATCTTGGGCTTGATCTGGTTTCCATAGAA
GTCAGCGCCATTGCAGACATCATTGCTGATTATGGGGACTCGAGCCTCCTGGAGTACCCCGGCCTGTTGG
CCATAGTACTGCGTGTTGCCCCAGCCCGTCACGGTACAGATCTTGCCATCCACCAGGGCCTGGCCGGCAG
CTGGGAGGCACACAGGCTGGATGTATTCTGTGAGGGGCAGGGGACTGGAGAGGTGGACCAGGGCAATATC
GTTGCTGTTCTCCTCGCTGTTGGGGTCCCGAAAGGGAAGATAGCCCCCGTGGTAGACCACAGCCTGCACC
CCCAGCTGCAGACCGTGGGGAGAGGCCTGGGCCACGGCACCGGCAAACACTCGCCATCGGGACAGGACCC
GGTTCCGCTCCGGCAAGCAGTGGGCGGCTGTCAGCACCCAGTCCCCGGAGAGCAGGGATCCCCCACAGAG
GTGTGCTCCATCATAGCGAAGGCTGACTTGCCACGGCCACCGGCCCAAGCTGGTGTCCCGGCCTCCCACG
ATGCGGTCCACGGGCAGCTTCCTGCGGCCACAGTCTTGGCAGATGGCGGCCAAGAAACGGCCTCTGGGGC
AATCACACACGGAGATGACCTCCAGCAGCCTCTGGGTGTGGGGCAGCCTCCCCTCGTCCACACAGAAGAA
GCCCGACGTGCCATTGGCGCCCGCCGTTCGCACGTCCAGCTCGGAGTGGGTCAGTGCCCTGAGGAAGCCC
ATCTCCTCGCAGCTGAGTCCGGCTACCCTGGCGTTGGAGCGCGAGGAGCACAGCAGCCGCCACGTCCCTT
CCGTCTTGTCAAAGACCATGAGCCGAGCGTCCGCAGAGCTGACCTGCACTGGGTACAGCGGCTCCTGGTC
ACTCCTGAGGAGAACAGCCACAATGGCCCAGGATGCCGCCCCGATGGCTGTCAGAAGTAGCAGGGTCCCC
GCAGTGAGAGCTGCCACCTTGGGTCTGGAGCAGCATGGCACAGTCCGGCCACCCTCCTTCTGCGCCATGT
CACTGCCTCTTGTTAATGATTCCCTGGCTGACCTCCTGGGCCAGGGTGGGACCTGTGAGGAGATGGACGG
GGAGGCAGGGCCTGGGGGAGCCCAGCCCAGCCCAGTCCTGGCGCCCCCAGTCCCAGGCGTCCATCCAGGC
AGGCTGTAGGGACTGGGCCTTGGCCAGAGCACGCCGTGATCACGGACGCAGATTGGGCTGGGTTCAAGGA
TGGGGTCAGTGTCTGACCAGCAGCGGGGGGACGCCTGGATTTGCAGGGATGGGGACCCCCCATGCCTGAGC
CTGGTGGAGCAGGGGACTGAGGATCCCGGTTTGAGGGAGGAGACAGCTGAGGACCTGAAATCATAAGTCT
TGGGAAAGGAGGAATTTGGGGGCCAGGACTCCCTAGTATGAGGGAGGAGGGGCCTGAGGGCTGGAACTCC
TGGGTCTGGGGAGGAAAGGACTGGGGTCCAACGGCTGAGTCTGAAGGAAGAGCAGGACAGAACACCTAGG
TGCTTGGGGAGACGTCATAGTGCCCCCTCTTCAGGTCCCCAGGAACCCCTCTATTAGGAGGTGGGCATTA
GGCTGGGTGGGGGGATGAGGGAACCCCTGTCCTCAGGGCTGGAACTGTGAGTCTGGGGGCCCTTGTCCTT
ACCCTGGGGTCCAGCAGGTGGGGGCGGAGCCTCGAGGTAGTGCCGGGGTCGGGTCAGTCTCCAGGCCTGG
GCAGGAGCATGGTGGCCCCGCAGCAGCGGGCGGCCTGGAGGCAGAGGCGGTGGCGTGGGGCCTGCTAGGC
CAGGCTGCCTCACCTGTGGGCCCTCAGGTAGGGTCCCTGGAAAGCGGGCTCGA

10

NOV8 is expressed in at least the following tissues: brain, Kidney, Liver, Lung, Ovary, Pancreas, Prostate, Stomach, Testis, Uterus, Whole embryo, kidney, and pancreas.

The disclosed NOV8 polypeptide (SEQ ID NO:40) encoded by SEQ ID NO:39 has 417 amino acid residues and is presented in Table 8B using the one-letter amino acid code.

15   Signal P, Psort and/or Hydropathy results predict that NOV8 has a signal peptide and is likely to be localized to the plasma membrane with a certainty of 0.7900. In other embodiments,

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NOV8 may also be localized to the microbody (peroxisome) with a certainty of 0.4294, the Golgi body with a certainty of 0.3000, or the endoplasmic reticulum (membrane) with a certainty of 0.2000. The most likely cleavage site is between positions 18 and 19: SWA-IV.

Table 20B. Encoded NOV8 protein sequence (SEQ ID NO:40).
MAQKEGGRTVPCCSRPKVAALTAGTLLLLTAIGAASWAIVAVLLRSDQEPLYPVQVSSAD ARLMVFDKTEGTWRLLCSSRSNARVAGLSCEEMGFLRALTHSELDVRTAGANGTSGFFCV DEGRLPHTQRLLEVISVCDCPRGRFLAAICQDCGRRKLPVDRIVGGRDTSLGRWPWQVSL RYDGAHLCGGSLLSGDWVLTAAHCFPERNRVLSRWRVFAGAVAQASPHGLQLGVQAVVYH GGYLPFRDPNSEENSNDIALVHLSSPLPLTEYIQPVCLPAAGQALVDGKICTVTGWGNTQ YYGQQAGVLQEARVPIISNDVCNGADFYGNQIKPKMFCAGYPEGGIDACQGDSGGPFVCE DSISRTPRWRLCGIVSWGTGCALAQKPGVYTKVSDFREWIFQAIKTHSEASGMVTQL

NOV8 has homology to the amino acid sequence shown in the BLASTP data listed in Table 20C.

Table 20C. BLAST results for NOV8					
Gene Index/ Identifier	Protein/ Organism	Length (aa)	Identity (%)	Positives (%)	Expect
ref|NP_002142.1| (NM_002151)	hepsin (transmembrane protease, serine 1); hepsin [Homo sapiens]	417	100	100	0.0
ref|NP_058808.1| (NM_017112)	hepsin [Rattus norvegicus]	416	88	91	0.0
ref|NP_032307.1| (NM_008281)	hepsin [Mus musculus]	416	88	92	0.0
emb|CAA30058.1| (X07002)	hepsin [Homo sapiens]	304	100	100	1e-179
dbj|BAB22289.1| (AK002694)	putative [Mus musculus]	502	73	77	1e-160

The homology of these sequences is shown graphically in the ClustalW analysis shown in Table 20D.

Table 20D. Information for the ClustalW proteins
1) NOV8 (SEQ ID NO: 40)
2) ref|NP_002142.1| (SEQ ID NO:136)
3) ref|NP_058808.1| (SEQ ID NO:137)
4) ref|NP_032307.1| (SEQ ID NO:138)
5) emb|CAA30058.1| (SEQ ID NO:139)
6) dbj|BAB22289.1| (SEQ ID NO:140)

```
                                    10        20        30        40        50
                           ....|....|....|....|....|....|....|....|....|....|....|
NOV8                       -----------------------------------------------------
ref|NP_002142.1|           -----------------------------------------------------
ref|NP_058808.1|           -----------------------------------------------------
ref|NP_032307.1|           -----------------------------------------------------
emb|CAA30058.1|            -----------------------------------------------------
dbj|BAB22289.1|            RPQLGRPHAAGCCCHPCLPGCPLLWGQTPCPCPGPETNPKPAPSPANPRV
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                          60        70        80        90        100
                   ....|....|....|....|....|....|....|....|....|....|
          NOV8                       ---------MAQKE--------------------GGRTVPCCS
          ref|NP_002142.1|           ---------MAQKE--------------------GGRTVPCCS
          ref|NP_058808.1|           ---------MAKE---------------------GGRTAPCCS
          ref|NP_032307.1|           ---------MAKE---------------------GGRTAACCS
          emb|CAA30058.1|            ------------------------------------------
          dbj|BAB22289.1|            PPQPNRSTWESLTRVPDMAKEDEEPGAHRGGSTCSRPQPGKGGRTAACCS

                          110       120       130       140       150
                   ....|....|....|....|....|....|....|....|....|....|
          NOV8               RPKVAALTAGTLLLLTAIGAASWAIVAVLLRSDQEPLYPVQMSSADARLM
          ref|NP_002142.1|   RPKVAALTAGTLLLLTAIGAASWAIVAVLLRSDQEPLYPVQMSSADARLM
          ref|NP_058808.1|   RPKVAALTVGTLLFLTGIGAASWAIVTLLLRSDQEPLYQVQLSPGDSRLL
          ref|NP_032307.1|   RPKVAALIVGTLLFLTGIGAASWAIVTLLLQSDQEPLYQVQLSPGDSRLA
          emb|CAA30058.1|    ------------------------------------------
          dbj|BAB22289.1|    RPKVAALIVGTLLFLTGIGAASWAIVTILLQSDQEPLYQVQLSPGDSRLA

                          160       170       180       190       200
                   ....|....|....|....|....|....|....|....|....|....|
          NOV8               VFDKTEGTWRLLCSSRSNARVAGLSCEEMGFLRALTHSELDVRTAGANGT
          ref|NP_002142.1|   VFDKTEGTWRLLCSSRSNARVAGLSCEEMGFLRALTHSELDVRTAGANGT
          ref|NP_058808.1|   VLDKTEGTWRLLCSSRSNARVAGLGCEEMGFLRALAHSELDVRTAGANGT
          ref|NP_032307.1|   VLDKTEGTWRLLCSSRSNARVAGLGCEEMGFLRALAHSELDVRTAGANGT
          emb|CAA30058.1|    ------------------------------------------T
          dbj|BAB22289.1|    VFDKTEGTWRLLCSSRSNARVAGLGCEEMGFLRALAHSELDVRTAGANGT

                          210       220       230       240       250
                   ....|....|....|....|....|....|....|....|....|....|
          NOV8               SGFFCVDEGRLPHTQRLLEVISVCDCPRGRFLAAICQDCGRRKLPVDRIV
          ref|NP_002142.1|   SGFFCVDEGRLPHTQRLLEVISVCDCPRGRFLAAICQDCGRRKLPVDRIV
          ref|NP_058808.1|   SGFFCVDEGGLPLAQRLLDVISVCDCPRGRFLTATCQDCGRRKLPVDRIV
          ref|NP_032307.1|   SGFFCVDEGGLPLAQRLLDVISVCDCPRGRFLTATCQDCGRRKLPVDRIV
          emb|CAA30058.1|    SGFFCVDEGRLPHTQRLLEVISVCDCPRGRFLAAICQDCGRRKLPVDRIV
          dbj|BAB22289.1|    SGFFCVDEGGLPLAQRLLDVISVCDCPRGRFLTATCQDCGRRSCRWTALW

                          260       270       280       290       300
                   ....|....|....|....|....|....|....|....|....|....|
          NOV8               GGRDTSLGRWPWQVSLRYDGAHLCGGSLLSGDWVLTAAHCFPERNRVLSR
          ref|NP_002142.1|   GGRDTSLGRWPWQVSLRYDGAHLCGGSLLSGDWVLTAAHCFPERNRVLSR
          ref|NP_058808.1|   GGQDSSLGRWPWQVSLRYDGTHLCGGSLLSGDWVLTAAHCFPERNRVLSR
          ref|NP_032307.1|   GGQDSSLGRWPWQVSLRYDGTHLCGGSLLSGDWVLTAAHCFPERNRVLSR
          emb|CAA30058.1|    GGRDTSLGRWPWQVSLRYDGAHLCGGSLLSGDWVLTAAHCFPERNRVLSR
          dbj|BAB22289.1|    GARTAVWEGGRGRSACVMMGTTSVGGPAVSG----LGADCCTLLSRAGDG

                          310       320       330       340       350
                   ....|....|....|....|....|....|....|....|....|....|
          NOV8               WRVFAGAVAQASP---HGTQLGVQAVVYHGGYLPFRDPNSEENSNDIALV
          ref|NP_002142.1|   WRVFAGAVAQASP---HGTQLGVQAVVYHGGYLPFRDPNSEENSNDIALV
          ref|NP_058808.1|   WRVFAGAVARTSP---HAVQLGVQAVYHGGYLPFRDPTIDENSNDIALV
          ref|NP_032307.1|   WRVFAGAVARTSP---HAVQLGVQAVYHGGYLPFRDPTIDENSNDIALV
          emb|CAA30058.1|    WRVFAGAVAQASP---HGTQLGVQAVVYHGGYLPFRDPNSEENSNDIALV
          dbj|BAB22289.1|    PVSVASICWCCSPDLTPCCATGVQAVTYHGGYLLFRDPTIDENSNDIALV

                          360       370       380       390       400
                   ....|....|....|....|....|....|....|....|....|....|
          NOV8               HLSSPLPLTEYIQPVCLPAAGQALVDGKTCTVTGWGNTQYYGQQAGVLQE
          ref|NP_002142.1|   HLSSPLPLTEYIQPVCLPAAGQALVDGKTCTVTGWGNTQYYGQQAGVLQE
          ref|NP_058808.1|   HLSSSLPLTEYIQPVCLPAAGQALVDGKVCTVTGWGNTQYYGQQAVVLQE
          ref|NP_032307.1|   HLSSSLPLTEYIQPVCLPAAGQALVDGKVCTVTGWGNTQYYGQQAMVLQE
          emb|CAA30058.1|    HLSSPLPLTEYIQPVCLPAAGQALVDGKTCTVTGWGNTQYYGQQAGVLQE
          dbj|BAB22289.1|    NLSSSLPLTEYIQPVCLPAAGQALVDGKVCTVTGWGNTQYYGQQAMVLQE

                          410       420       430       440       450
                   ....|....|....|....|....|....|....|....|....|....|
          NOV8               ARVPIISNDVCNGADFYGNQIKPKMFCAGYPEGGIDACQGDSGGPFVCED
          ref|NP_002142.1|   ARVPIISNDVCNGADFYGNQIKPKMFCAGYPEGGIDACQGDSGGPFVCED
          ref|NP_058808.1|   ARVPIISNEVCNSPDFYGNQIKPKMFCAGYPEGGIDACQGDSCGHFVCED
          ref|NP_032307.1|   ARVPIISNEVCNSPDFYGNQIKPKMFCAGYPEGGIDACQGDSGGPFVCED
          emb|CAA30058.1|    ARVPIISNDVCNGADFYGNQIKPKMFCAGYPEGGIDACQGDSGGPFVCED
          dbj|BAB22289.1|    ARVPIISNEVCNSPDFYGNQIKPKMFCAGYPEGGIDACQGDSGGPFVCED

                          460       470       480       490       500
                   ....|....|....|....|....|....|....|....|....|....|
          NOV8               SISRTPRWRLCGIVSWGTGCALAQKPGVYTKVSDFREWIFQAIKTHSEAS
          ref|NP_002142.1|   SISRTPRWRLCGIVSWGTGCALAQKPGVYTKVSDFREWIFQAIKTHSEAS
          ref|NP_058808.1|   RISGTSRWRLCGIVSWGTGCALARKPGVYTKVIDFREWIFQAIKTHSEAT
          ref|NP_032307.1|   SISGTSRWRLCGIVSWGTGCALARKPGVYTKVIDFREWIFQAIKTHSEAS
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emb|CAA30058.1|   SISRIPRWRLCGIVSWGTGCALAQKPGVYTKVSDFREWIFQAIKTHSEAS
dbj|BAB22289.1|   SISGTSRWRLCGIVSWGTGCALARKPGVYTKVEDFREWIFRAIKTHSEAS
```

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                  ....|.
NOV8              GMVTQL
ref|NP_002142.1|  GMVTQL
ref|NP_058808.1|  GMVTQP
ref|NP_032307.1|  GMVTQP
emb|CAA30058.1|   GMVTQL
dbj|BAB22289.1|   GMVTQP
```

Tables 20E lists the domain description from DOMAIN analysis results against NOV8.
This indicates that the NOV8 sequence has properties similar to those of other proteins known
to contain this domain.

Table 20E. Domain Analysis of NOV8
gnl|Smart|smart00020, Tryp_SPc, Trypsin-like serine protease CD-Length = 230 residues, 100.0% aligned   Score = 261 bits (668), Expect = 4e-71

Hepsin is a type II transmembrane serine protease abundantly expressed on the surface
of hepatocytes. Biochemical studies have shown that hepsin is an enzyme of 51 kDa with the
trypsin-like substrate specificity. Several in vitro studies have suggested that hepsin may play
a role in blood coagulation, hepatocyte growth, and fertilization. To determine the functional
importance of hepsin, hepsin-deficient mice were generated by homologous recombination.
Homozygous hepsin-/- mice were viable and fertile, and grew normally. When analyzed in
hemostasis assays, such as tail bleeding time and plasma clotting times, and in vivo modes,
such as disseminated intravascular coagulation, septic shock, and acute liver regeneration,
hepsin-/- mice had similar phenotypes as wild-type controls. Liver weight and serum
concentrations of liver-derived proteins or enzymes were also similar in hepsin-/- and wild-
type mice. No abnormalities were identified in major organs in hepsin-/- mice in histological
examinations. These results indicate that hepsin is not an essential enzyme for normal
hemostasis, embryogenesis, and maintenance of normal liver function. Unexpectedly, serum
concentrations of bone-derived alkaline phosphatase were approximately two-fold higher in
both male and female hepsin-/- mice than those in wild-type controls. The underlying
mechanism for this phenotype and long-term effects of hepsin deficiency remain to be
determined. (See Wu, 2001, Front Biosci 1;6:D192-200).

The disclosed NOV8 nucleic acid of the invention encoding a hepsin-like protein
includes the nucleic acid whose sequence is provided in Table 20A, or a fragment thereof.
The invention also includes a mutant or variant nucleic acid any of whose bases may be
changed from the corresponding base shown in Table 20A while still encoding a protein that

maintains its hepsin-like activities and physiological functions, or a fragment of such a nucleic

acid. The invention further includes nucleic acids whose sequences are complementary to

those just described, including nucleic acid fragments that are complementary to any of the

nucleic acids just described. The invention additionally includes nucleic acids or nucleic acid

5   fragments, or complements thereto, whose structures include chemical modifications. Such

modifications include, by way of nonlimiting example, modified bases, and nucleic acids

whose sugar phosphate backbones are modified or derivatized. These modifications are

carried out at least in part to enhance the chemical stability of the modified nucleic acid, such

that they may be used, for example, as antisense binding nucleic acids in therapeutic

10   applications in a subject. In the mutant or variant nucleic acids, and their complements, up to

about 0 percent of the bases may be so changed.

The disclosed NOV8 protein of the invention includes the hepsin-like protein whose

sequence is provided in Table 20B. The invention also includes a mutant or variant protein

any of whose residues may be changed from the corresponding residue shown in Table 2 while

15   still encoding a protein that maintains its hepsin-like activities and physiological functions, or

a functional fragment thereof. In the mutant or variant protein, up to about 0 percent of the

residues may be so changed.

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or

$(F_{ab})_2$, that bind immunospecifically to any of the proteins of the invention.

20   The above defined information for this invention suggests that this hepsin-like protein

(NOV8) may function as a member of a "RNase family". Therefore, the NOV8 nucleic acids

and proteins identified here may be useful in potential therapeutic applications implicated in

(but not limited to) various pathologies and disorders as indicated below. The potential

therapeutic applications for this invention include, but are not limited to: protein therapeutic,

25   small molecule drug target, antibody target (therapeutic, diagnostic, drug targeting/cytotoxic

antibody), diagnostic and/or prognostic marker, gene therapy (gene delivery/gene ablation),

research tools, tissue regeneration *in vivo* and *in vitro* of all tissues and cell types composing

(but not limited to) those defined here.

The NOV8 nucleic acids and proteins of the invention are useful in potential

30   therapeutic applications implicated in cancer, especially prostate and ovarian cancer, and/or

other pathologies/disorders.

NOV8 nucleic acids and polypeptides are further useful in the generation of antibodies

that bind immuno-specifically to the novel NOV8 substances for use in therapeutic or

diagnostic methods. These antibodies may be generated according to methods known in the

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art, using prediction from hydrophobicity charts, as described in the "Anti-NOVX Antibodies" section below. The disclosed NOV8 protein has multiple hydrophilic regions, each of which can be used as an immunogen. These novel proteins can be used in assay systems for functional analysis of various human disorders, which will help in understanding of pathology

5   of the disease and development of new drug targets for various disorders.

SECX and/or NOVX Nucleic Acids and Polypeptides

One aspect of the invention pertains to isolated nucleic acid molecules that encode SECX and/or NOVX polypeptides or biologically active portions thereof. Also included in the invention are nucleic acid fragments sufficient for use as hybridization probes to identify

10   SECX and/or NOVX-encoding nucleic acids (*e.g.*, SECX and/or NOVX mRNAs) and fragments for use as PCR primers for the amplification and/or mutation of SECX and/or NOVX nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments

15   and homologs thereof. The nucleic acid molecule may be single-stranded or double-stranded, but preferably is comprised double-stranded DNA.

An SECX and/or NOVX nucleic acid can encode a mature SECX and/or NOVX polypeptide. As used herein, a "mature" form of a polypeptide or protein disclosed in the present invention is the product of a naturally occurring polypeptide or precursor form or

20   proprotein. The naturally occurring polypeptide, precursor or proprotein includes, by way of nonlimiting example, the full-length gene product, encoded by the corresponding gene. Alternatively, it may be defined as the polypeptide, precursor or proprotein encoded by an ORF described herein. The product "mature" form arises, again by way of nonlimiting example, as a result of one or more naturally occurring processing steps as they may take place

25   within the cell, or host cell, in which the gene product arises. Examples of such processing steps leading to a "mature" form of a polypeptide or protein include the cleavage of the N-terminal methionine residue encoded by the initiation codon of an ORF, or the proteolytic cleavage of a signal peptide or leader sequence. Thus a mature form arising from a precursor polypeptide or protein that has residues 1 to N, where residue 1 is the N-terminal methionine,

30   would have residues 2 through N remaining after removal of the N-terminal methionine. Alternatively, a mature form arising from a precursor polypeptide or protein having residues 1 to N, in which an N-terminal signal sequence from residue 1 to residue M is cleaved, would have the residues from residue M+1 to residue N remaining. Further as used herein, a

"mature" form of a polypeptide or protein may arise from a step of post-translational modification other than a proteolytic cleavage event. Such additional processes include, by way of non-limiting example, glycosylation, myristoylation or phosphorylation. In general, a mature polypeptide or protein may result from the operation of only one of these processes, or

5   a combination of any of them.

The term "probes", as utilized herein, refers to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as approximately, *e.g.*, 6,000 nt, depending upon the specific use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length

10   probes are generally obtained from a natural or recombinant source, are highly specific, and much slower to hybridize than shorter-length oligomer probes. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

The term "isolated" nucleic acid molecule, as utilized herein, is one, which is separated

15   from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5'- and 3'-termini of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated SECX and/or NOVX nucleic acid molecules can contain less than about 5 kb, 4

20   kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell/tissue from which the nucleic acid is derived (*e.g.*, brain, heart, liver, spleen, etc.). Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when

25   chemically synthesized.

A nucleic acid molecule of the invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, or a complement of this aforementioned nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein.

30   Using all or a portion of the nucleic acid sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39 as a hybridization probe, SECX and/or NOVX molecules can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook, *et al.*, (eds.), MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et*

al., (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard

5   PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to SECX and/or NOVX nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide

10   residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or

15   100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment of the invention, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at least 6 contiguous nucleotides SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, or a complement thereof. Oligonucleotides may be chemically synthesized and may also be used as probes.

20   In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, or a portion of this nucleotide sequence (*e.g.*, a fragment that can be used as a probe or primer or a fragment encoding a biologically-active portion of an SECX and/or NOVX polypeptide). A nucleic

25   acid molecule that is complementary to the nucleotide sequence shown SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 or 39 is one that is sufficiently complementary to the nucleotide sequence shown SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 or 39 that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19,

30   21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, thereby forming a stable duplex.

As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, van

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der Waals, hydrophobic interactions, and the like. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical

5   intermediates.

Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence.

10   Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side

15   chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type. Homologs are nucleic acid sequences or amino acid sequences of a particular gene that are derived from different species.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or

20   analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 70%, 80%, or 95% identity (with a preferred identity of 80-95%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a

25   computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. *See e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below.

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or

30   variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of SECX and/or NOVX polypeptides. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the invention,

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homologous nucleotide sequences include nucleotide sequences encoding for an SECX and/or NOVX polypeptide of species other than humans, including, but not limited to: vertebrates, and thus can include, *e.g.*, frog, mouse, rat, rabbit, dog, cat cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring

5   allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the exact nucleotide sequence encoding human SECX and/or NOVX protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, as well as a

10   polypeptide possessing SECX and/or NOVX biological activity. Various biological activities of the SECX and/or NOVX proteins are described below.

An SECX and/or NOVX polypeptide is encoded by the open reading frame ("ORF") of an SECX and/or NOVX nucleic acid. An ORF corresponds to a nucleotide sequence that could potentially be translated into a polypeptide. A stretch of nucleic acids comprising an ORF is

15   uninterrupted by a stop codon. An ORF that represents the coding sequence for a full protein begins with an ATG "start" codon and terminates with one of the three "stop" codons, namely, TAA, TAG, or TGA. For the purposes of this invention, an ORF may be any part of a coding sequence, with or without a start codon, a stop codon, or both. For an ORF to be considered as a good candidate for coding for a *bona fide* cellular protein, a minimum size requirement is

20   often set, *e.g.*, a stretch of DNA that would encode a protein of 50 amino acids or more.

The nucleotide sequences determined from the cloning of the human SECX and/or NOVX genes allows for the generation of probes and primers designed for use in identifying and/or cloning SECX and/or NOVX homologues in other cell types, *e.g.* from other tissues, as well as SECX and/or NOVX homologues from other vertebrates. The probe/primer typically

25   comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 consecutive sense strand nucleotide sequence SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 or 39; or an anti-sense strand nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23,

30   25, 27, 29, 31, 33, 35, 37 or 39; or of a naturally occurring mutant of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39.

Probes based on the human SECX and/or NOVX nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.* the label group

can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissues which mis-express an SECX and/or NOVX protein, such as by measuring a level of an SECX and/or NOVX-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting SECX and/or

5   NOVX mRNA levels or determining whether a genomic SECX and/or NOVX gene has been mutated or deleted.

"A polypeptide having a biologically-active portion of an SECX and/or NOVX polypeptide" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the invention, including mature forms, as measured in a

10   particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically-active portion of SECX and/or NOVX" can be prepared by isolating a portion SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 or 39, that encodes a polypeptide having an SECX and/or NOVX biological activity (the biological activities of the SECX and/or NOVX proteins are described below), expressing the encoded

15   portion of SECX and/or NOVX protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of SECX and/or NOVX.

SECX and/or NOVX Nucleic Acid and Polypeptide Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27,

20   29, 31, 33, 35, 37 and 39 due to degeneracy of the genetic code and thus encode the same SECX and/or NOVX proteins as that encoded by the nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12,

25   14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40.

In addition to the human SECX and/or NOVX nucleotide sequences shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the SECX and/or NOVX polypeptides may exist within a

30   population (*e.g.*, the human population). Such genetic polymorphism in the SECX and/or NOVX genes may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame (ORF) encoding an SECX and/or NOVX protein,

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preferably a vertebrate SECX and/or NOVX protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the SECX and/or NOVX genes. Any and all such nucleotide variations and resulting amino acid polymorphisms in the SECX and/or NOVX polypeptides, which are the result of natural allelic variation and that do not

5   alter the functional activity of the SECX and/or NOVX polypeptides, are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding SECX and/or NOVX proteins from other species, and thus that have a nucleotide sequence that differs from the human SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39 are intended to be within

10   the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the SECX and/or NOVX cDNAs of the invention can be isolated based on their homology to the human SECX and/or NOVX nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions.

15   Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500, 750, 1000, 1500, or 2000 or more nucleotides in

20   length. In yet another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

25   Homologs (i.e., nucleic acids encoding SECX and/or NOVX proteins derived from species other than human) or other related sequences (e.g., paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

30   As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5 °C lower than the

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thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm,

5   50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at

pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and

10   oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

       Stringent conditions are known to those skilled in the art and can be found in Ausubel, *et al.*, (eds.), CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%,

15   70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions are hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C, followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated

20   nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequences SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

25   In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution,

30   0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well-known within the art. *See, e.g.*, Ausubel, et al. (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990; GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequences SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization

5   conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (e.g., as employed for cross-species hybridizations). See,

10   e.g., Ausubel, et al. (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981. *Proc Natl Acad Sci USA* **78**: 6789-6792.

15   **Conservative Mutations**

In addition to naturally-occurring allelic variants of SECX and/or NOVX sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, thereby leading to changes in the amino acid

20   sequences of the encoded SECX and/or NOVX proteins, without altering the functional ability of said SECX and/or NOVX proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40. A "non-essential" amino acid residue is a residue that can be altered from the wild-type

25   sequences of the SECX and/or NOVX proteins without altering their biological activity, whereas an "essential" amino acid residue is required for such biological activity. For example, amino acid residues that are conserved among the SECX and/or NOVX proteins of the invention are predicted to be particularly non-amenable to alteration. Amino acids for which conservative substitutions can be made are well-known within the art.

30   Another aspect of the invention pertains to nucleic acid molecules encoding SECX and/or NOVX proteins that contain changes in amino acid residues that are not essential for activity. Such SECX and/or NOVX proteins differ in amino acid sequence from SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39 yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a

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nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 45% homologous to the amino acid sequences SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 and 40. Preferably, the protein encoded by the nucleic acid molecule is at least about 60% homologous to SEQ ID NOS:2, 4,

5   6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 and 40; more preferably at least about 70% homologous SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40; still more preferably at least about 80% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40; even more preferably at least about 90% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30,

10   32, 34, 36, 38 or 40; and most preferably at least about 95% homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40.

An isolated nucleic acid molecule encoding an SECX and/or NOVX protein homologous to the protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40 can be created by introducing one or more nucleotide substitutions,

15   additions or deletions into the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39 by standard techniques, such as site-directed mutagenesis and

20   PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted, non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined within the art. These families include amino acids with basic side chains (*e.g.*,

25   lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted

30   non-essential amino acid residue in the SECX and/or NOVX protein is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of an SECX and/or NOVX coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for SECX and/or NOVX biological activity to identify mutants that retain activity. Following

mutagenesis SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

The relatedness of amino acid families may also be determined based on side chain interactions. Substituted amino acids may be fully conserved "strong" residues or fully conserved "weak" residues. The "strong" group of conserved amino acid residues may be any one of the following groups: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW, wherein the single letter amino acid codes are grouped by those amino acids that may be substituted for each other. Likewise, the "weak" group of conserved residues may be any one of the following: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY, wherein the letters within each group represent the single letter amino acid code.

In one embodiment, a mutant SECX and/or NOVX protein can be assayed for (*i*) the ability to form protein:protein interactions with other SECX and/or NOVX proteins, other cell-surface proteins, or biologically-active portions thereof, (*ii*) complex formation between a mutant SECX and/or NOVX protein and an SECX and/or NOVX ligand; or (*iii*) the ability of a mutant SECX and/or NOVX protein to bind to an intracellular target protein or biologically-active portion thereof; (*e.g.* avidin proteins).

In yet another embodiment, a mutant SECX and/or NOVX protein can be assayed for the ability to regulate a specific biological function (*e.g.*, regulation of insulin release).

Antisense Nucleic Acids

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein (*e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence). In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire SECX and/or NOVX coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of an SECX and/or NOVX protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40, or antisense nucleic acids complementary to an SECX and/or NOVX nucleic

acid sequence of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding an SECX and/or NOVX protein. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding the SECX and/or NOVX protein. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding the SECX and/or NOVX protein disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of SECX and/or NOVX mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of SECX and/or NOVX mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of SECX and/or NOVX mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally-occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids (*e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used).

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil,

queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a
5   nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or
10   genomic DNA encoding an SECX and/or NOVX protein to thereby inhibit expression of the protein (*e.g.*, by inhibiting transcription and/or translation). The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration
15   of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface (*e.g.*, by linking the antisense nucleic acid molecules to peptides or
20   antibodies that bind to cell surface receptors or antigens). The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient nucleic acid molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an
25   α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other. *See, e.g.,* Gaultier, *et al.*, 1987. *Nucl. Acids Res.* **15**: 6625-6641. The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (*See, e.g.,* Inoue, *et al.* 1987. *Nucl. Acids Res.* **15**: 6131-6148) or a
30   chimeric RNA-DNA analogue (*See, e.g.,* Inoue, *et al.*, 1987. *FEBS Lett.* **215**: 327-330.

Ribozymes and PNA Moieties

Nucleic acid modifications include, by way of non-limiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified

5   nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

In one embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a

10   complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes as described in Haselhoff and Gerlach 1988. *Nature* 334: 585-591) can be used to catalytically cleave SECX and/or NOVX mRNA transcripts to thereby inhibit translation of SECX and/or NOVX mRNA. A ribozyme having specificity for an SECX and/or NOVX-encoding nucleic acid can be designed based upon the nucleotide sequence of an SECX and/or NOVX cDNA disclosed

15   herein (*i.e.*, SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an SECX and/or NOVX-encoding mRNA. *See, e.g.*, U.S. Patent 4,987,071 to Cech, *et al.* and U.S. Patent 5,116,742 to Cech, *et al.* SECX and/or NOVX mRNA can also be

20   used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. *See, e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, SECX and/or NOVX gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the SECX and/or NOVX nucleic acid (*e.g.*, the SECX and/or NOVX promoter and/or enhancers) to form triple helical

25   structures that prevent transcription of the SECX and/or NOVX gene in target cells. *See, e.g.*, Helene, 1991. *Anticancer Drug Des.* 6: 569-84; Helene, *et al.* 1992. *Ann. N.Y. Acad. Sci.* 660: 27-36; Maher, 1992. *Bioassays* 14: 807-15.

In various embodiments, the SECX and/or NOVX nucleic acids can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization,

30   or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids. *See, e.g.*, Hyrup, *et al.*, 1996. *Bioorg Med Chem* 4: 5-23. As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics (*e.g.*, DNA mimics) in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The

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neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup, *et al.*, 1996. *supra*; Perry-O'Keefe, *et al.*, 1996. *Proc. Natl. Acad. Sci. USA* 93: 14670-14675.

5   PNAs of SECX and/or NOVX can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of SECX and/or NOVX can also be used, for example, in the analysis of single base pair mutations in a gene (*e.g.*, PNA directed PCR clamping; as artificial
10   restriction enzymes when used in combination with other enzymes, *e.g.*, S$_1$ nucleases (*See,* Hyrup, *et al.*, 1996.*supra*); or as probes or primers for DNA sequence and hybridization (*See,* Hyrup, *et al.*, 1996, *supra*; Perry-O'Keefe, *et al.*, 1996. *supra*).

In another embodiment, PNAs of SECX and/or NOVX can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to
15   PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of SECX and/or NOVX can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes (*e.g.*, RNase H and DNA polymerases) to interact with the DNA portion while the PNA portion would provide high binding affinity and
20   specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (*see,* Hyrup, et al., 1996. *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup, *et al.*, 1996. *supra* and Finn, *et al.*, 1996. *Nucl Acids Res* 24: 3357-3363. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling
25   chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA. *See, e.g.,* Mag, *et al.*, 1989. *Nucl Acid Res* 17: 5973-5988. PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment. *See, e.g.,* Finn, *et al.*, 1996. *supra*. Alternatively,
30   chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. *See, e.g.,* Petersen, *et al.*, 1975. *Bioorg. Med. Chem. Lett.* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (*see, e.g.,* Letsinger, *et al.*, 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86:

6553-6556; Lemaitre, *et al.*, 1987. *Proc. Natl. Acad. Sci.* 84: 648-652; PCT Publication No. WO88/09810) or the blood-brain barrier (*see, e.g.*, PCT Publication No. WO 89/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (*see, e.g.*, Krol, *et al.*, 1988. *BioTechniques* 6:958-976) or intercalating agents (*see, e.g.*, Zon, 1988.

5   *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, and the like.

SECX and/or NOVX Polypeptides

A polypeptide according to the invention includes a polypeptide including the amino

10   acid sequence of SECX and/or NOVX polypeptides whose sequences are provided in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residues shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40 while still encoding a protein that maintains its SECX and/or NOVX

15   activities and physiological functions, or a functional fragment thereof.

In general, an SECX and/or NOVX variant that preserves SECX and/or NOVX-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the

20   possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above.

One aspect of the invention pertains to isolated SECX and/or NOVX proteins, and biologically-active portions thereof, or derivatives, fragments, analogs or homologs thereof.

25   Also provided are polypeptide fragments suitable for use as immunogens to raise anti-SECX and/or NOVX antibodies. In one embodiment, native SECX and/or NOVX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, SECX and/or NOVX proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, an SECX

30   and/or NOVX protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" polypeptide or protein or biologically-active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue

source from which the SECX and/or NOVX protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of SECX and/or NOVX proteins in which the protein is separated from cellular components of the cells from which it is

5   isolated or recombinantly-produced. In one embodiment, the language "substantially free of cellular material" includes preparations of SECX and/or NOVX proteins having less than about 30% (by dry weight) of non-SECX and/or NOVX proteins (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-SECX and/or NOVX proteins, still more preferably less than about 10% of non-SECX and/or NOVX proteins, and

10   most preferably less than about 5% of non-SECX and/or NOVX proteins. When the SECX and/or NOVX protein or biologically-active portion thereof is recombinantly-produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the SECX and/or NOVX protein preparation.

15   The language "substantially free of chemical precursors or other chemicals" includes preparations of SECX and/or NOVX proteins in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of SECX and/or NOVX proteins having less than about 30% (by dry

20   weight) of chemical precursors or non-SECX and/or NOVX chemicals, more preferably less than about 20% chemical precursors or non-SECX and/or NOVX chemicals, still more preferably less than about 10% chemical precursors or non-SECX and/or NOVX chemicals, and most preferably less than about 5% chemical precursors or non-SECX and/or NOVX chemicals.

25   Biologically-active portions of SECX and/or NOVX proteins include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequences of the SECX and/or NOVX proteins (e.g., the amino acid sequence shown in SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40) that include fewer amino acids than the full-length SECX and/or NOVX proteins, and exhibit at least one

30   activity of an SECX and/or NOVX protein. Typically, biologically-active portions comprise a domain or motif with at least one activity of the SECX and/or NOVX protein. A biologically-active portion of an SECX and/or NOVX protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acid residues in length.

Moreover, other biologically-active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native SECX and/or NOVX protein.

In an embodiment, the SECX and/or NOVX protein has an amino acid sequence

5   shown SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40. In other embodiments, the SECX and/or NOVX protein is substantially homologous to SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40, and retains the functional activity of the protein of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40, yet differs in amino acid sequence due to natural allelic

10   variation or mutagenesis, as described in detail, below. Accordingly, in another embodiment, the SECX and/or NOVX protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40, and retains the functional activity of the SECX and/or NOVX proteins of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32,

15   34, 36, 38 or 40.

Determining Homology Between Two or More Sequences

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced

20   in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that

25   position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. *See,* Needleman and

30   Wunsch, 1970. *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or

99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of

5   comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.,* A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.,*

10   the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a

15   reference sequence over a comparison region.

Chimeric and Fusion Proteins

The invention also provides SECX and/or NOVX chimeric or fusion proteins. As used herein, an SECX and/or NOVX "chimeric protein" or "fusion protein" comprises an SECX

20   and/or NOVX polypeptide operatively-linked to a non-SECX and/or NOVX polypeptide. An "SECX and/or NOVX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to an SECX and/or NOVX protein SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 or 40, whereas a "non-SECX and/or NOVX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not

25   substantially homologous to the SECX and/or NOVX protein, *e.g.,* a protein that is different from the SECX and/or NOVX protein and that is derived from the same or a different organism. Within an SECX and/or NOVX fusion protein the SECX and/or NOVX polypeptide can correspond to all or a portion of an SECX and/or NOVX protein. In one embodiment, an SECX and/or NOVX fusion protein comprises at least one biologically-active

30   portion of an SECX and/or NOVX protein. In another embodiment, an SECX and/or NOVX fusion protein comprises at least two biologically-active portions of an SECX and/or NOVX protein. In yet another embodiment, an SECX and/or NOVX fusion protein comprises at least three biologically-active portions of an SECX and/or NOVX protein. Within the fusion protein, the term "operatively-linked" is intended to indicate that the SECX and/or NOVX

polypeptide and the non-SECX and/or NOVX polypeptide are fused in-frame with one another. The non-SECX and/or NOVX polypeptide can be fused to the N-terminus or C-terminus of the SECX and/or NOVX polypeptide.

In one embodiment, the fusion protein is a GST-SECX and/or NOVX fusion protein in which the SECX and/or NOVX sequences are fused to the C-terminus of the GST (glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant SECX and/or NOVX polypeptides.

In another embodiment, the fusion protein is an SECX and/or NOVX protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of SECX and/or NOVX can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is an SECX and/or NOVX-immunoglobulin fusion protein in which the SECX and/or NOVX sequences are fused to sequences derived from a member of the immunoglobulin protein family. The SECX and/or NOVX-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between an SECX and/or NOVX ligand and an SECX and/or NOVX protein on the surface of a cell, to thereby suppress SECX and/or NOVX-mediated signal transduction *in vivo*. The SECX and/or NOVX-immunoglobulin fusion proteins can be used to affect the bioavailability of an SECX and/or NOVX cognate ligand. Inhibition of the SECX and/or NOVX ligand/SECX and/or NOVX interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (*e.g.* promoting or inhibiting) cell survival. Moreover, the SECX and/or NOVX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-SECX and/or NOVX antibodies in a subject, to purify SECX and/or NOVX ligands, and in screening assays to identify molecules that inhibit the interaction of SECX and/or NOVX with an SECX and/or NOVX ligand.

An SECX and/or NOVX chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be

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carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g.,* Ausubel, *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially

5   available that already encode a fusion moiety (*e.g.,* a GST polypeptide). An SECX and/or NOVX-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the SECX and/or NOVX protein.

SECX and/or NOVX Agonists and Antagonists

10   The invention also pertains to variants of the SECX and/or NOVX proteins that function as either SECX and/or NOVX agonists (*i.e.,* mimetics) or as SECX and/or NOVX antagonists. Variants of the SECX and/or NOVX protein can be generated by mutagenesis (*e.g.,* discrete point mutation or truncation of the SECX and/or NOVX protein). An agonist of the SECX and/or NOVX protein can retain substantially the same, or a subset of, the

15   biological activities of the naturally occurring form of the SECX and/or NOVX protein. An antagonist of the SECX and/or NOVX protein can inhibit one or more of the activities of the naturally occurring form of the SECX and/or NOVX protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the SECX and/or NOVX protein. Thus, specific biological effects can be elicited by treatment

20   with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the SECX and/or NOVX proteins.

Variants of the SECX and/or NOVX proteins that function as either SECX and/or

25   NOVX agonists (*i.e.,* mimetics) or as SECX and/or NOVX antagonists can be identified by screening combinatorial libraries of mutants (*e.g.,* truncation mutants) of the SECX and/or NOVX proteins for SECX and/or NOVX protein agonist or antagonist activity. In one embodiment, a variegated library of SECX and/or NOVX variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene

30   library. A variegated library of SECX and/or NOVX variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential SECX and/or NOVX sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.,* for phage display) containing the set of SECX and/or NOVX sequences therein. There are a variety of

methods which can be used to produce libraries of potential SECX and/or NOVX variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the

5   provision, in one mixture, of all of the sequences encoding the desired set of potential SECX and/or NOVX sequences. Methods for synthesizing degenerate oligonucleotides are well-known within the art. *See, e.g.,* Narang, 1983. *Tetrahedron* 39: 3; Itakura, *et al.,* 1984. *Annu. Rev. Biochem.* 53: 323; Itakura, *et al.,* 1984. *Science* 198: 1056; Ike, *et al.,* 1983. *Nucl. Acids Res.* 11: 477.

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Polypeptide Libraries

In addition, libraries of fragments of the SECX and/or NOVX protein coding sequences can be used to generate a variegated population of SECX and/or NOVX fragments for screening and subsequent selection of variants of an SECX and/or NOVX protein. In one

15   embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of an SECX and/or NOVX coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double-stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from

20   reformed duplexes by treatment with S_1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, expression libraries can be derived which encodes N-terminal and internal fragments of various sizes of the SECX and/or NOVX proteins.

Various techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene

25   products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of SECX and/or NOVX proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and

30   expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify

SECX and/or NOVX variants. *See, e.g.*, Arkin and Yourvan, 1992. *Proc. Natl. Acad. Sci. USA* 89: 7811-7815; Delgrave, *et al.*, 1993. *Protein Engineering* 6:327-331.

SECX and/or NOVX Antibodies

The term "antibody" as used herein refers to immunoglobulin molecules and

5   immunologically active portions of immunoglobulin (Ig) molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab}, $F_{ab'}$ and $F_{(ab')2}$ fragments, and an F_{ab} expression library. In general, antibody molecules obtained from humans relates to any of the classes IgG, IgM, IgA, IgE and IgD, which differ

10   from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG_1, IgG_2, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of human antibody species.

An isolated protein of the invention intended to serve as an antigen, or a portion or

15   fragment thereof, can be used as an immunogen to generate antibodies that immunospecifically bind the antigen, using standard techniques for polyclonal and monoclonal antibody preparation. The full-length protein can be used or, alternatively, the invention provides antigenic peptide fragments of the antigen for use as immunogens. An antigenic peptide fragment comprises at least 6 amino acid residues of the amino acid sequence of the

20   full length protein, such as an amino acid sequence shown in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 and 40, and encompasses an epitope thereof such that an antibody raised against the peptide forms a specific immune complex with the full length protein or with any fragment that contains the epitope. Preferably, the antigenic peptide comprises at least 10 amino acid residues, or at least 15 amino acid residues, or at least 20

25   amino acid residues, or at least 30 amino acid residues. Preferred epitopes encompassed by the antigenic peptide are regions of the protein that are located on its surface; commonly these are hydrophilic regions.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region of SECX and/or NOVX that is located on the surface of the

30   protein, *e.g.*, a hydrophilic region. A hydrophobicity analysis of the human SECX and/or NOVX protein sequence will indicate which regions of a SECX and/or NOVX polypeptide are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots

showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, *e.g.*, Hopp and Woods, 1981, *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle 1982, *J. Mol. Biol.* 157: 105-142, each

5   incorporated herein by reference in their entirety. Antibodies that are specific for one or more domains within an antigenic protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

A protein of the invention, or a derivative, fragment, analog, homolog or ortholog thereof, may be utilized as an immunogen in the generation of antibodies that

10   immunospecifically bind these protein components.

Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies directed against a protein of the invention, or against derivatives, fragments, analogs homologs or orthologs thereof (see, for example, Antibodies: A Laboratory Manual, Harlow E, and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring

15   Harbor, NY, incorporated herein by reference). Some of these antibodies are discussed below.

Polyclonal Antibodies

For the production of polyclonal antibodies, various suitable host animals (e.g., rabbit, goat, mouse or other mammal) may be immunized by one or more injections with the native protein, a synthetic variant thereof, or a derivative of the foregoing. An appropriate

20   immunogenic preparation can contain, for example, the naturally occurring immunogenic protein, a chemically synthesized polypeptide representing the immunogenic protein, or a recombinantly expressed immunogenic protein. Furthermore, the protein may be conjugated to a second protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum

25   albumin, bovine thyroglobulin, and soybean trypsin inhibitor. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (e.g., aluminum hydroxide), surface active substances (e.g., lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), adjuvants usable in humans such as Bacille

30   Calmette-Guerin and Corynebacterium parvum, or similar immunostimulatory agents. Additional examples of adjuvants which can be employed include MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate).

The polyclonal antibody molecules directed against the immunogenic protein can be isolated from the mammal (e.g., from the blood) and further purified by well known

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techniques, such as affinity chromatography using protein A or protein G, which provide primarily the IgG fraction of immune serum. Subsequently, or alternatively, the specific antigen which is the target of the immunoglobulin sought, or an epitope thereof, may be immobilized on a column to purify the immune specific antibody by immunoaffinity

5   chromatography. Purification of immunoglobulins is discussed, for example, by D. Wilkinson (The Scientist, published by The Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28).

Monoclonal Antibodies

10   The term "monoclonal antibody" (MAb) or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs thus

15   contain an antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

Monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to

20   elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized in vitro.

The immunizing agent will typically include the protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human

25   mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are

30   employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas

typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a
5   medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, J. Immunol., 133:3001 (1984); Brodeur
10   et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by
15   immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980). It is an objective, especially important in therapeutic applications of monoclonal antibodies, to
20   identify antibodies having a high degree of specificity and a high binding affinity for the target antigen.

After the desired hybridoma cells are identified, the clones can be subcloned by limiting dilution procedures and grown by standard methods (Goding,1986). Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-
25   1640 medium. Alternatively, the hybridoma cells can be grown in vivo as ascites in a mammal.

The monoclonal antibodies secreted by the subclones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel
30   electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies can also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the

heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to

5   obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, Nature 368, 812-13 (1994)) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin

10   polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

Humanized Antibodies

15   The antibodies directed against the protein antigens of the invention can further comprise humanized antibodies or human antibodies. These antibodies are suitable for administration to humans without engendering an immune response by the human against the administered immunoglobulin. Humanized forms of antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')$_2$ or other antigen-

20   binding subsequences of antibodies) that are principally comprised of the sequence of a human immunoglobulin, and contain minimal sequence derived from a non-human immunoglobulin. Humanization can be performed following the method of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the

25   corresponding sequences of a human antibody. (See also U.S. Patent No. 5,225,539.) In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically

30   two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that

of a human immunoglobulin (Jones et al., 1986; Riechmann et al., 1988; and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)).

Human Antibodies

5   Fully human antibodies essentially relate to antibody molecules in which the entire sequence of both the light chain and the heavy chain, including the CDRs, arise from human genes. Such antibodies are termed "human antibodies", or "fully human antibodies" herein. Human monoclonal antibodies can be prepared by the trioma technique; the human B-cell hybridoma technique (see Kozbor, et al., 1983 Immunol Today 4: 72) and the EBV hybridoma

10   technique to produce human monoclonal antibodies (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus in vitro (see Cole, et al., 1985 In:

15   MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96).

         In addition, human antibodies can also be produced using additional techniques, including phage display libraries (Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the

20   endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in Marks et al. (Bio/Technology 10, 779-783 (1992));

25   Lonberg et al. (Nature 368 856-859 (1994)); Morrison (Nature 368, 812-13 (1994)); Fishwild et al,(Nature Biotechnology 14, 845-51 (1996)); Neuberger (Nature Biotechnology 14, 826 (1996)); and Lonberg and Huszar (Intern. Rev. Immunol. 13 65-93 (1995)).

         Human antibodies may additionally be produced using transgenic nonhuman animals which are modified so as to produce fully human antibodies rather than the animal's

30   endogenous antibodies in response to challenge by an antigen. (See PCT publication WO94/02602). The endogenous genes encoding the heavy and light immunoglobulin chains in the nonhuman host have been incapacitated, and active loci encoding human heavy and light chain immunoglobulins are inserted into the host's genome. The human genes are incorporated, for example, using yeast artificial chromosomes containing the requisite human

DNA segments. An animal which provides all the desired modifications is then obtained as progeny by crossbreeding intermediate transgenic animals containing fewer than the full complement of the modifications. The preferred embodiment of such a nonhuman animal is a mouse, and is termed the Xenomouse™ as disclosed in PCT publications WO 96/33735 and

5   WO 96/34096. This animal produces B cells which secrete fully human immunoglobulins. The antibodies can be obtained directly from the animal after immunization with an immunogen of interest, as, for example, a preparation of a polyclonal antibody, or alternatively from immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with human

10   variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs of antibodies such as, for example, single chain Fv molecules.

An example of a method of producing a nonhuman host, exemplified as a mouse, lacking expression of an endogenous immunoglobulin heavy chain is disclosed in U.S. Patent

15   No. 5,939,598. It can be obtained by a method including deleting the J segment genes from at least one endogenous heavy chain locus in an embryonic stem cell to prevent rearrangement of the locus and to prevent formation of a transcript of a rearranged immunoglobulin heavy chain locus, the deletion being effected by a targeting vector containing a gene encoding a selectable marker; and producing from the embryonic stem cell a transgenic mouse whose somatic and

20   germ cells contain the gene encoding the selectable marker.

A method for producing an antibody of interest, such as a human antibody, is disclosed in U.S. Patent No. 5,916,771. It includes introducing an expression vector that contains a nucleotide sequence encoding a heavy chain into one mammalian host cell in culture, introducing an expression vector containing a nucleotide sequence encoding a light chain into

25   another mammalian host cell, and fusing the two cells to form a hybrid cell. The hybrid cell expresses an antibody containing the heavy chain and the light chain.

In a further improvement on this procedure, a method for identifying a clinically relevant epitope on an immunogen, and a correlative method for selecting an antibody that binds immunospecifically to the relevant epitope with high affinity, are disclosed in PCT

30   publication WO 99/53049.

F$_{ab}$ Fragments and Single Chain Antibodies

155

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an antigenic protein of the invention (see e.g., U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F_{ab} expression libraries (see e.g., Huse, et al., 1989 Science 246: 1275-1281) to allow rapid and effective

5   identification of monoclonal F_{ab} fragments with the desired specificity for a protein or derivatives, fragments, analogs or homologs thereof. Antibody fragments that contain the idiotypes to a protein antigen may be produced by techniques known in the art including, but not limited to: (i) an $F_{(ab')2}$ fragment produced by pepsin digestion of an antibody molecule; (ii) an F_{ab} fragment generated by reducing the disulfide bridges of an $F_{(ab')2}$ fragment; (iii) an F_{ab}

10   fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) F_v fragments.

Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that

15   have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for an antigenic protein of the invention. The second binding target is any other antigen, and advantageously is a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two

20   immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by

25   affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., EMBO J., 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part

30   of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-

transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which
5   are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing
10   large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')$_2$ bispecific antibodies). Techniques for generating bispecific antibodies from
15   antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')$_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab'
20   fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.
25   Additionally, Fab' fragments can be directly recovered from E. coli and chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med. 175:217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')$_2$ molecule. Each Fab' fragment was separately secreted from E. coli and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to
30   cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol. 148(5):1547-1553

(1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber et al., J. Immunol. 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al., J. Immunol. 147:60 (1991).

Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in the protein antigen of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin molecule can be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the protein antigen described herein and further binds tissue factor (TF).

Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360; WO 92/200373; EP 03089). It is contemplated that the antibodies can be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by

forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

5   **Effector Function Engineering**

It can be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, e.g., the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have

10   improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., J. Exp Med., 176: 1191-1195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity can also be prepared using heterobifunctional cross-linkers as described in Wolff et al. Cancer Research, 53: 2560-2565 (1993). Alternatively, an antibody

15   can be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., Anti-Cancer Drug Design, 3: 219-230 (1989).

Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated

20   to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used

25   include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of

30   radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl

adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-

5   2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody can be conjugated to a "receptor" (such

10   streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) that is in turn conjugated to a cytotoxic agent.

15   **Immunoliposomes**

The antibodies disclosed herein can also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang et al., Proc. Natl Acad. Sci. USA, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545.

20   Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody

25   of the present invention can be conjugated to the liposomes as described in Martin et al ., J. Biol. Chem., 257: 286-288 (1982) via a disulfide-interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon et al., J. National Cancer Inst., 81(19): 1484 (1989).

30   **Diagnostic Applications of Antibodies Directed Against the Proteins of the Invention**

Antibodies directed against a protein of the invention may be used in methods known within the art relating to the localization and/or quantitation of the protein (e.g., for use in measuring levels of the protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies

against the proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antigen binding domain, are utilized as pharmacologically-active compounds (see below).

An antibody specific for a protein of the invention can be used to isolate the protein by standard techniques, such as immunoaffinity chromatography or immunoprecipitation. Such
5   an antibody can facilitate the purification of the natural protein antigen from cells and of recombinantly produced antigen expressed in host cells. Moreover, such an antibody can be used to detect the antigenic protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the antigenic protein. Antibodies directed against the protein can be used diagnostically to monitor protein levels in tissue as part of a
10   clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline
15   phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include
20   luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

Antibody Therapeutics

25   Antibodies of the invention, including polyclonal, monoclonal, humanized and fully human antibodies, may used as therapeutic agents. Such agents will generally be employed to treat or prevent a disease or pathology in a subject. An antibody preparation, preferably one having high specificity and high affinity for its target antigen, is administered to the subject and will generally have an effect due to its binding with the target. Such an effect may be one
30   of two kinds, depending on the specific nature of the interaction between the given antibody molecule and the target antigen in question. In the first instance, administration of the antibody may abrogate or inhibit the binding of the target with an endogenous ligand to which it naturally binds. In this case, the antibody binds to the target and masks a binding site of the

naturally occurring ligand, wherein the ligand serves as an effector molecule. Thus the receptor mediates a signal transduction pathway for which ligand is responsible.

Alternatively, the effect may be one in which the antibody elicits a physiological result by virtue of binding to an effector binding site on the target molecule. In this case the target, a
5   receptor having an endogenous ligand which may be absent or defective in the disease or pathology, binds the antibody as a surrogate effector ligand, initiating a receptor-based signal transduction event by the receptor.

A therapeutically effective amount of an antibody of the invention relates generally to the amount needed to achieve a therapeutic objective. As noted above, this may be a binding
10   interaction between the antibody and its target antigen that, in certain cases, interferes with the functioning of the target, and in other cases, promotes a physiological response. The amount required to be administered will furthermore depend on the binding affinity of the antibody for its specific antigen, and will also depend on the rate at which an administered antibody is depleted from the free volume other subject to which it is administered. Common ranges for
15   therapeutically effective dosing of an antibody or antibody fragment of the invention may be, by way of nonlimiting example, from about 0.1 mg/kg body weight to about 50 mg/kg body weight. Common dosing frequencies may range, for example, from twice daily to once a week.

20   **Pharmaceutical Compositions of Antibodies**

Antibodies specifically binding a protein of the invention, as well as other molecules identified by the screening assays disclosed herein, can be administered for the treatment of various disorders in the form of pharmaceutical compositions. Principles and considerations involved in preparing such compositions, as well as guidance in the choice of components are
25   provided, for example, in Remington : The Science And Practice Of Pharmacy 19th ed. (Alfonso R. Gennaro, et al., editors) Mack Pub. Co., Easton, Pa. : 1995; Drug Absorption Enhancement : Concepts, Possibilities, Limitations, And Trends, Harwood Academic Publishers, Langhorne, Pa., 1994; and Peptide And Protein Drug Delivery (Advances In Parenteral Sciences, Vol. 4), 1991, M. Dekker, New York.

30   If the antigenic protein is intracellular and whole antibodies are used as inhibitors, internalizing antibodies are preferred. However, liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody, peptide

molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. See, e.g., Marasco et al., Proc. Natl. Acad. Sci. USA, 90: 7889-7893 (1993). The formulation herein can also contain more than one active compound as necessary for the particular

5   indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition can comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

10   The active ingredients can also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in

15   macroemulsions.

The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers

20   containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON

25   DEPOT ™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

30   **ELISA Assay**

An agent for detecting an analyte protein is an antibody capable of binding to an analyte protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., F_{ab} or $F_{(ab)2}$) can be used. The term "labeled", with regard to the probe or antibody, is intended to

encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary

5   antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. Included within the usage of the term "biological sample", therefore, is blood and a fraction or component of blood including blood serum, blood plasma, or lymph.

10   That is, the detection method of the invention can be used to detect an analyte mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of an analyte mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of an analyte protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and

15   immunofluorescence. *In vitro* techniques for detection of an analyte genomic DNA include Southern hybridizations. Procedures for conducting immunoassays are described, for example in "ELISA: Theory and Practice: Methods in Molecular Biology", Vol. 42, J. R. Crowther (Ed.) Human Press, Totowa, NJ, 1995; "Immunoassay", E. Diamandis and T. Christopoulus, Academic Press, Inc., San Diego, CA, 1996; and "Practice and Thory of Enzyme

20   Immunoassays", P. Tijssen, Elsevier Science Publishers, Amsterdam, 1985. Furthermore, *in vivo* techniques for detection of an analyte protein include introducing into a subject a labeled anti-an analyte protein antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

25

SECX and/or NOVX Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding an SECX and/or NOVX protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid

30   molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors

having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are

5 operatively-linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective

10 retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid

15 sequence to be expressed. Within a recombinant expression vector, "operably-linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell).

20 The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell

25 and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including

30 fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, SECX and/or NOVX proteins, mutant forms of SECX and/or NOVX proteins, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of SECX and/or NOVX proteins in prokaryotic or eukaryotic cells. For example, SECX and/or NOVX proteins can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using

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baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory

5   sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors

10   typically serve three purposes: (*i*) to increase expression of recombinant protein; (*ii*) to increase the solubility of the recombinant protein; and (*iii*) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety

15   subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the

20   target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

25   One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. *See, e.g.,* Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the

30   individual codons for each amino acid are those preferentially utilized in *E. coli* (*see, e.g.,* Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the SECX and/or NOVX expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerivisae*

include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and

Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz *et al.*, 1987. *Gene* 54: 113-123),

pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego,

Calif.).

5   Alternatively, SECX and/or NOVX can be expressed in insect cells using baculovirus

expression vectors. Baculovirus vectors available for expression of proteins in cultured insect

cells (*e.g.*, SF9 cells) include the pAc series (Smith, *et al.*, 1983. *Mol. Cell. Biol.* 3:

2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian

10   cells using a mammalian expression vector. Examples of mammalian expression vectors

include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, *et al.*, 1987. *EMBO*

J. 6: 187-195). When used in mammalian cells, the expression vector's control functions are

often provided by viral regulatory elements. For example, commonly used promoters are

derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable

15   expression systems for both prokaryotic and eukaryotic cells see, *e.g.*, Chapters 16 and 17 of

Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring

Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of

directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*,

20   tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific

regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific

promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, 1987. *Genes Dev.* 1:

268-277), lymphoid-specific promoters (Calame and Eaton, 1988. *Adv. Immunol.* 43:

235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989. *EMBO J.*

25   8: 729-733) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and

Baltimore, 1983. *Cell* 33: 741-748), neuron-specific promoters (*e.g.*, the neurofilament

promoter; Byrne and Ruddle, 1989. *Proc. Natl. Acad. Sci. USA* 86: 5473-5477),

pancreas-specific promoters (Edlund, *et al.*, 1985. *Science* 230: 912-916), and mammary

gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European

30   Application Publication No. 264,166). Developmentally-regulated promoters are also

encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, 1990. *Science* 249: 374-379)

and the α-fetoprotein promoter (Campes and Tilghman, 1989. *Genes Dev.* 3: 537-546).

The invention further provides a recombinant expression vector comprising a DNA

molecule of the invention cloned into the expression vector in an antisense orientation. That

is, the DNA molecule is operatively-linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to SECX and/or NOVX mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes *see, e.g.,* Weintraub, *et al.,* "Antisense RNA as a molecular tool for genetic analysis," *Reviews-Trends in Genetics,* Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, SECX and/or NOVX protein can be expressed in bacterial cells such as *E. coli,* insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.,* DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate

the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a

5   selectable marker can be introduced into a host cell on the same vector as that encoding SECX and/or NOVX or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can

10   be used to produce (*i.e.*, express) SECX and/or NOVX protein. Accordingly, the invention further provides methods for producing SECX and/or NOVX protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding SECX and/or NOVX protein has been introduced) in a suitable medium such that SECX and/or NOVX protein is produced. In

15   another embodiment, the method further comprises isolating SECX and/or NOVX protein from the medium or the host cell.

Transgenic SECX and/or NOVX Animals

The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or

20   an embryonic stem cell into which SECX and/or NOVX protein-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous SECX and/or NOVX sequences have been introduced into their genome or homologous recombinant animals in which endogenous SECX and/or NOVX sequences have been altered. Such animals are useful for studying the function and/or activity of SECX

25   and/or NOVX protein and for identifying and/or evaluating modulators of SECX and/or NOVX protein activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is

30   exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal,

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preferably a mammal, more preferably a mouse, in which an endogenous SECX and/or NOVX gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

5   A transgenic animal of the invention can be created by introducing SECX and/or NOVX-encoding nucleic acid into the male pronuclei of a fertilized oocyte (*e.g.*, by microinjection, retroviral infection) and allowing the oocyte to develop in a pseudopregnant female foster animal. The human SECX and/or NOVX cDNA sequences SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39 can be introduced as a

10   transgene into the genome of a non-human animal. Alternatively, a non-human homologue of the human SECX and/or NOVX gene, such as a mouse SECX and/or NOVX gene, can be isolated based on hybridization to the human SECX and/or NOVX cDNA (described further *supra*) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A

15   tissue-specific regulatory sequence(s) can be operably-linked to the SECX and/or NOVX transgene to direct expression of SECX and/or NOVX protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan, 1986. In: MANIPULATING

20   THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the SECX and/or NOVX transgene in its genome and/or expression of SECX and/or NOVX mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene.

25   Moreover, transgenic animals carrying a transgene-encoding SECX and/or NOVX protein can further be bred to other transgenic animals carrying other transgenes.

          To create a homologous recombinant animal, a vector is prepared which contains at least a portion of an SECX and/or NOVX gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the SECX and/or NOVX gene.

30   The SECX and/or NOVX gene can be a human gene (*e.g.*, the cDNA of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39), but more preferably, is a non-human homologue of a human SECX and/or NOVX gene. For example, a mouse homologue of human SECX and/or NOVX gene of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39 can be used to construct a homologous

recombination vector suitable for altering an endogenous SECX and/or NOVX gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous SECX and/or NOVX gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

5   Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous SECX and/or NOVX gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous SECX and/or NOVX protein). In the homologous recombination vector, the altered portion of the SECX and/or NOVX gene is flanked at its 5'-

10   and 3'-termini by additional nucleic acid of the SECX and/or NOVX gene to allow for homologous recombination to occur between the exogenous SECX and/or NOVX gene carried by the vector and an endogenous SECX and/or NOVX gene in an embryonic stem cell. The additional flanking SECX and/or NOVX nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of

15   flanking DNA (both at the 5'- and 3'-termini) are included in the vector. *See, e.g.*, Thomas, *et al.*, 1987. *Cell* 51: 503 for a description of homologous recombination vectors. The vector is ten introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced SECX and/or NOVX gene has homologously-recombined with the endogenous SECX and/or NOVX gene are selected. *See, e.g.*, Li, *et al.*, 1992. *Cell* 69: 915.

20   The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. *See, e.g.*, Bradley, 1987. In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously-recombined DNA in

25   their germ cells can be used to breed animals in which all cells of the animal contain the homologously-recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley, 1991. *Curr. Opin. Biotechnol.* 2: 823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

30   In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, *See, e.g.*, Lakso, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 6232-6236. Another example of a recombinase system is the FLP recombinase system of

Saccharomyces cerevisiae. See, O'Gorman, *et al.,* 1991. *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.,* by

5   mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, *et al.,* 1997. *Nature* 385: 810-813. In brief, a cell (*e.g.,* a somatic cell) from the transgenic animal can be isolated and induced to exit the

10   growth cycle and enter G_0 phase. The quiescent cell can then be fused, *e.g.,* through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell

15   (*e.g.,* the somatic cell) is isolated.

Pharmaceutical Compositions

The SECX and/or NOVX nucleic acid molecules, SECX and/or NOVX proteins, and anti-SECX and/or NOVX antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into

20   pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration.

25   Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for

30   pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral,

5   intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates,

10   and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous

15   solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under

20   the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by

25   the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the

30   composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, an SECX and/or NOVX protein or anti-SECX and/or NOVX antibody) in the required amount

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in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile

5   powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic

10   administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules,

15   troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint,

20   methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For

25   transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into

30   ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

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In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides,

5   polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared

10   according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated;

15   each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for

20   the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (*see, e.g.,* U.S. Patent No. 5,328,470) or by stereotactic injection (*see, e.g.,* Chen, *et al.,* 1994. *Proc. Natl. Acad. Sci. USA* 91: 3054-3057).

25   The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.,* retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

30   The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Screening and Detection Methods

The isolated nucleic acid molecules of the invention can be used to express SECX and/or NOVX protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect SECX and/or NOVX mRNA (*e.g.*, in a biological sample) or a genetic

5   lesion in an SECX and/or NOVX gene, and to modulate SECX and/or NOVX activity, as described further, below. In addition, the SECX and/or NOVX proteins can be used to screen drugs or compounds that modulate the SECX and/or NOVX protein activity or expression as well as to treat disorders characterized by insufficient or excessive production of SECX and/or NOVX protein or production of SECX and/or NOVX protein forms that have decreased or

10   aberrant activity compared to SECX and/or NOVX wild-type protein (*e.g.*; diabetes (regulates insulin release); obesity (binds and transport lipids); metabolic disturbances associated with obesity, the metabolic syndrome X as well as anorexia and wasting disorders associated with chronic diseases and various cancers, and infectious disease(possesses anti-microbial activity) and the various dyslipidemias. In addition, the anti-SECX and/or NOVX antibodies of the

15   invention can be used to detect and isolate SECX and/or NOVX proteins and modulate SECX and/or NOVX activity. In yet a further aspect, the invention can be used in methods to influence appetite, absorption of nutrients and the disposition of metabolic substrates in both a positive and negative fashion.

The invention further pertains to novel agents identified by the screening assays

20   described herein and uses thereof for treatments as described, *supra*.

Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides,

25   peptidomimetics, small molecules or other drugs) that bind to SECX and/or NOVX proteins or have a stimulatory or inhibitory effect on, *e.g.*, SECX and/or NOVX protein expression or SECX and/or NOVX protein activity. The invention also includes compounds identified in the screening assays described herein.

In one embodiment, the invention provides assays for screening candidate or test

30   compounds which bind to or modulate the activity of the membrane-bound form of an SECX and/or NOVX protein or polypeptide or biologically-active portion thereof. The test compounds of the invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring

deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds. *See, e.g.,* Lam, 1997. *Anticancer Drug Design* 12:

5   145.

A "small molecule" as used herein, is meant to refer to a composition that has a molecular weight of less than about 5 kD and most preferably less than about 4 kD. Small molecules can be, *e.g.*, nucleic acids, peptides, polypeptides, peptidomimetics, carbohydrates, lipids or other organic or inorganic molecules. Libraries of chemical and/or biological

10   mixtures, such as fungal, bacterial, or algal extracts, are known in the art and can be screened with any of the assays of the invention.

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt, *et al.*, 1993. *Proc. Natl. Acad. Sci. U.S.A.* 90: 6909; Erb, *et al.*, 1994. *Proc. Natl. Acad. Sci. U.S.A.* 91: 11422; Zuckermann, *et al.*, 1994. *J. Med. Chem.* 37: 2678;

15   Cho, *et al.*, 1993. *Science* 261: 1303; Carrell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2059; Carell, *et al.*, 1994. *Angew. Chem. Int. Ed. Engl.* 33: 2061; and Gallop, *et al.*, 1994. *J. Med. Chem.* 37: 1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten, 1992. *Biotechniques* 13: 412-421), or on beads (Lam, 1991. *Nature* 354: 82-84), on chips (Fodor,

20   1993. *Nature* 364: 555-556), bacteria (Ladner, U.S. Patent No. 5,223,409), spores (Ladner, U.S. Patent 5,233,409), plasmids (Cull, *et al.*, 1992. *Proc. Natl. Acad. Sci. USA* 89: 1865-1869) or on phage (Scott and Smith, 1990. *Science* 249: 386-390; Devlin, 1990. *Science* 249: 404-406; Cwirla, *et al.*, 1990. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6378-6382; Felici, 1991. *J. Mol. Biol.* 222: 301-310; Ladner, U.S. Patent No. 5,233,409.).

25   In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of SECX and/or NOVX protein, or a biologically-active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to an SECX and/or NOVX protein determined. The cell, for example, can of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to

30   the SECX and/or NOVX protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the SECX and/or NOVX protein or biologically-active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct

counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically-labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which

5   expresses a membrane-bound form of SECX and/or NOVX protein, or a biologically-active portion thereof, on the cell surface with a known compound which binds SECX and/or NOVX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an SECX and/or NOVX protein, wherein determining the ability of the test compound to interact with an SECX and/or NOVX protein

10   comprises determining the ability of the test compound to preferentially bind to SECX and/or NOVX protein or a biologically-active portion thereof as compared to the known compound.

       In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of SECX and/or NOVX protein, or a biologically-active portion thereof, on the cell surface with a test compound and determining the ability of the test

15   compound to modulate (e.g., stimulate or inhibit) the activity of the SECX and/or NOVX protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of SECX and/or NOVX or a biologically-active portion thereof can be accomplished, for example, by determining the ability of the SECX and/or NOVX protein to bind to or interact with an SECX and/or NOVX target molecule. As used herein, a "target

20   molecule" is a molecule with which an SECX and/or NOVX protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses an SECX and/or NOVX interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. An SECX and/or NOVX target molecule can be a non-SECX and/or

25   NOVX molecule or an SECX and/or NOVX protein or polypeptide of the invention. In one embodiment, an SECX and/or NOVX target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (e.g. a signal generated by binding of a compound to a membrane-bound SECX and/or NOVX molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that

30   has catalytic activity or a protein that facilitates the association of downstream signaling molecules with SECX and/or NOVX.

       Determining the ability of the SECX and/or NOVX protein to bind to or interact with an SECX and/or NOVX target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the SECX

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and/or NOVX protein to bind to or interact with an SECX and/or NOVX target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+}, diacylglycerol, IP_3, etc.), detecting

5   catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising an SECX and/or NOVX-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the invention is a cell-free assay comprising

10   contacting an SECX and/or NOVX protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to bind to the SECX and/or NOVX protein or biologically-active portion thereof. Binding of the test compound to the SECX and/or NOVX protein can be determined either directly or indirectly as described above. In one such embodiment, the assay comprises contacting the SECX and/or NOVX

15   protein or biologically-active portion thereof with a known compound which binds SECX and/or NOVX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an SECX and/or NOVX protein, wherein determining the ability of the test compound to interact with an SECX and/or NOVX protein comprises determining the ability of the test compound to preferentially bind to

20   SECX and/or NOVX or biologically-active portion thereof as compared to the known compound.

In still another embodiment, an assay is a cell-free assay comprising contacting SECX and/or NOVX protein or biologically-active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.* stimulate or inhibit) the activity

25   of the SECX and/or NOVX protein or biologically-active portion thereof. Determining the ability of the test compound to modulate the activity of SECX and/or NOVX can be accomplished, for example, by determining the ability of the SECX and/or NOVX protein to bind to an SECX and/or NOVX target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test

30   compound to modulate the activity of SECX and/or NOVX protein can be accomplished by determining the ability of the SECX and/or NOVX protein further modulate an SECX and/or NOVX target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as described, *supra*.

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In yet another embodiment, the cell-free assay comprises contacting the SECX and/or NOVX protein or biologically-active portion thereof with a known compound which binds SECX and/or NOVX protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with an SECX

5 and/or NOVX protein, wherein determining the ability of the test compound to interact with an SECX and/or NOVX protein comprises determining the ability of the SECX and/or NOVX protein to preferentially bind to or modulate the activity of an SECX and/or NOVX target molecule.

The cell-free assays of the invention are amenable to use of both the soluble form or

10 the membrane-bound form of SECX and/or NOVX protein. In the case of cell-free assays comprising the membrane-bound form of SECX and/or NOVX protein, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of SECX and/or NOVX protein is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside,

15 octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, N-dodecyl--
N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl) dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

20 In more than one embodiment of the above assay methods of the invention, it may be desirable to immobilize either SECX and/or NOVX protein or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to SECX and/or NOVX protein, or interaction of SECX and/or NOVX protein with a target molecule in the presence

25 and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-SECX and/or NOVX fusion proteins or GST-target fusion proteins can be adsorbed onto

30 glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or SECX and/or NOVX protein, and the mixture is incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to

remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described, *supra*. Alternatively, the complexes can be dissociated from the matrix, and the level of SECX and/or NOVX protein binding or activity determined using standard techniques.

5   Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the SECX and/or NOVX protein or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated SECX and/or NOVX protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well-known within the art (*e.g.*,

10   biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with SECX and/or NOVX protein or target molecules, but which do not interfere with binding of the SECX and/or NOVX protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or SECX and/or NOVX protein trapped in the wells by antibody

15   conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the SECX and/or NOVX protein or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the SECX and/or NOVX protein or target molecule.

20   In another embodiment, modulators of SECX and/or NOVX protein expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of SECX and/or NOVX mRNA or protein in the cell is determined. The level of expression of SECX and/or NOVX mRNA or protein in the presence of the candidate compound is compared to the level of expression of SECX and/or NOVX mRNA or protein in

25   the absence of the candidate compound. The candidate compound can then be identified as a modulator of SECX and/or NOVX mRNA or protein expression based upon this comparison. For example, when expression of SECX and/or NOVX mRNA or protein is greater (*i.e.*, statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of SECX and/or NOVX mRNA

30   or protein expression. Alternatively, when expression of SECX and/or NOVX mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of SECX and/or NOVX mRNA or protein expression. The level of SECX and/or NOVX mRNA or protein expression

in the cells can be determined by methods described herein for detecting SECX and/or NOVX mRNA or protein.

In yet another aspect of the invention, the SECX and/or NOVX proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (see, e.g., U.S. Patent No.

5   5,283,317; Zervos, et al., 1993. Cell 72: 223-232; Madura, et al., 1993. J. Biol. Chem. 268: 12046-12054; Bartel, et al., 1993. Biotechniques 14: 920-924; Iwabuchi, et al., 1993. Oncogene 8: 1693-1696; and Brent WO 94/10300), to identify other proteins that bind to or interact with SECX and/or NOVX ("SECX and/or NOVX-binding proteins" or "SECX and/or NOVX-bp") and modulate SECX and/or NOVX activity. Such SECX and/or NOVX-binding

10   proteins are also likely to be involved in the propagation of signals by the SECX and/or NOVX proteins as, for example, upstream or downstream elements of the SECX and/or NOVX pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes

15   two different DNA constructs. In one construct, the gene that codes for SECX and/or NOVX is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are

20   able to interact, in vivo, forming an SECX and/or NOVX-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription

25   factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with SECX and/or NOVX.

The invention further pertains to novel agents identified by the aforementioned screening assays and uses thereof for treatments as described herein.

Detection Assays

30   Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. By way of example, and not of limitation, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii)

identify an individual from a minute biological sample (tissue typing); and (*iii*) aid in forensic
identification of a biological sample. Some of these applications are described in the
subsections, below.

5   **Chromosome Mapping**

Once the sequence (or a portion of the sequence) of a gene has been isolated, this
sequence can be used to map the location of the gene on a chromosome. This process is called
chromosome mapping. Accordingly, portions or fragments of the SECX and/or NOVX
sequences, SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and

10   39, or fragments or derivatives thereof, can be used to map the location of the SECX and/or
NOVX genes, respectively, on a chromosome. The mapping of the SECX and/or NOVX
sequences to chromosomes is an important first step in correlating these sequences with genes
associated with disease.

Briefly, SECX and/or NOVX genes can be mapped to chromosomes by preparing PCR

15   primers (preferably 15-25 bp in length) from the SECX and/or NOVX sequences. Computer
analysis of the SECX and/or NOVX, sequences can be used to rapidly select primers that do
not span more than one exon in the genomic DNA, thus complicating the amplification
process. These primers can then be used for PCR screening of somatic cell hybrids containing
individual human chromosomes. Only those hybrids containing the human gene

20   corresponding to the SECX and/or NOVX sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals
(*e.g.*, human and mouse cells). As hybrids of human and mouse cells grow and divide, they
gradually lose human chromosomes in random order, but retain the mouse chromosomes. By
using media in which mouse cells cannot grow, because they lack a particular enzyme, but in

25   which human cells can, the one human chromosome that contains the gene encoding the
needed enzyme will be retained. By using various media, panels of hybrid cell lines can be
established. Each cell line in a panel contains either a single human chromosome or a small
number of human chromosomes, and a full set of mouse chromosomes, allowing easy
mapping of individual genes to specific human chromosomes. *See, e.g.*, D'Eustachio, *et al.*,

30   1983. *Science* 220: 919-924. Somatic cell hybrids containing only fragments of human
chromosomes can also be produced by using human chromosomes with translocations and
deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular
sequence to a particular chromosome. Three or more sequences can be assigned per day using

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a single thermal cycler. Using the SECX and/or NOVX sequences to design oligonucleotide primers, sub-localization can be achieved with panels of fragments from specific chromosomes.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase

5   chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical like colcemid that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually.

10   The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases, will suffice to get good results at a reasonable amount of time. For a review of this technique, *see,* Verma, *et al.,* HUMAN CHROMOSOMES: A

15   MANUAL OF BASIC TECHNIQUES (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more

20   likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, *e.g.,* in McKusick, MENDELIAN INHERITANCE IN MAN, available on-line

25   through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, *e.g.,* Egeland, *et al.,* 1987. *Nature,* 325: 783-787.

Moreover, differences in the DNA sequences between individuals affected and

30   unaffected with a disease associated with the SECX and/or NOVX gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible

from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

5   **Tissue Typing**

The SECX and/or NOVX sequences of the invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the invention are useful as additional DNA

10   markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Patent No. 5,272,057).

Furthermore, the sequences of the invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the SECX and/or NOVX sequences described herein can be used

15   to prepare two PCR primers from the 5'- and 3'-termini of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the invention can be used to

20   obtain such identification sequences from individuals and from tissue. The SECX and/or NOVX sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic

25   variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are

30   necessary to differentiate individuals. The noncoding sequences can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39 are used,

a more appropriate number of primers for positive individual identification would be 500-2,000.

Predictive Medicine

5   The invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the invention relates to diagnostic assays for determining SECX and/or NOVX protein and/or nucleic acid expression as well as SECX and/or NOVX activity, in the context

10 of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant SECX and/or NOVX expression or activity. The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder,

15 immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with SECX and/or NOVX protein, nucleic acid expression or activity.

20 For example, mutations in an SECX and/or NOVX gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with SECX and/or NOVX protein, nucleic acid expression, or biological activity.

   Another aspect of the invention provides methods for determining SECX and/or

25 NOVX protein, nucleic acid expression or activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to

30 respond to a particular agent.)

   Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of SECX and/or NOVX in clinical trials.

   These and other agents are described in further detail in the following sections.

Diagnostic Assays

An exemplary method for detecting the presence or absence of SECX and/or NOVX in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting SECX and/or NOVX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes SECX and/or NOVX protein such that the presence of SECX and/or NOVX is detected in the biological sample. An agent for detecting SECX and/or NOVX mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to SECX and/or NOVX mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length SECX and/or NOVX nucleic acid, such as the nucleic acid of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to SECX and/or NOVX mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting SECX and/or NOVX protein is an antibody capable of binding to SECX and/or NOVX protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')$_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect SECX and/or NOVX mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of SECX and/or NOVX mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of SECX and/or NOVX protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of SECX and/or NOVX genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of SECX and/or NOVX protein include

introducing into a subject a labeled anti-SECX and/or NOVX antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting SECX and/or NOVX protein, mRNA, or genomic DNA, such that the presence of SECX and/or NOVX protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of SECX and/or NOVX protein, mRNA or genomic DNA in the control sample with the presence of SECX and/or NOVX protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of SECX and/or NOVX in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting SECX and/or NOVX protein or mRNA in a biological sample; means for determining the amount of SECX and/or NOVX in the sample; and means for comparing the amount of SECX and/or NOVX in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect SECX and/or NOVX protein or nucleic acid.

Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant SECX and/or NOVX expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with SECX and/or NOVX protein, nucleic acid expression or activity. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the invention provides a method for identifying a disease or disorder associated with aberrant SECX and/or NOVX expression or activity in which a test sample is obtained from a subject and SECX and/or NOVX protein or nucleic acid (e.g., mRNA, genomic DNA) is detected, wherein the presence of SECX and/or NOVX protein or nucleic acid is diagnostic for a subject having or

at risk of developing a disease or disorder associated with aberrant SECX and/or NOVX expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

5   Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant SECX and/or NOVX expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent

10   for a disorder. Thus, the invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant SECX and/or NOVX expression or activity in which a test sample is obtained and SECX and/or NOVX protein or nucleic acid is detected (*e.g.*, wherein the presence of SECX and/or NOVX protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated

15   with aberrant SECX and/or NOVX expression or activity).

The methods of the invention can also be used to detect genetic lesions in an SECX and/or NOVX gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In various embodiments, the methods include detecting, in a sample of cells from the subject, the

20   presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding an SECX and/or NOVX-protein, or the misexpression of the SECX and/or NOVX gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of: (*i*) a deletion of one or more nucleotides from an SECX and/or NOVX gene; (*ii*) an addition of one or more nucleotides to an SECX and/or NOVX gene; (*iii*)

25   a substitution of one or more nucleotides of an SECX and/or NOVX gene, (*iv*) a chromosomal rearrangement of an SECX and/or NOVX gene; (*v*) an alteration in the level of a messenger RNA transcript of an SECX and/or NOVX gene, (*vi*) aberrant modification of an SECX and/or NOVX gene, such as of the methylation pattern of the genomic DNA, (*vii*) the presence of a non-wild-type splicing pattern of a messenger RNA transcript of an SECX and/or NOVX

30   gene, (*viii*) a non-wild-type level of an SECX and/or NOVX protein, (*ix*) allelic loss of an SECX and/or NOVX gene, and (*x*) inappropriate post-translational modification of an SECX and/or NOVX protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in an SECX and/or NOVX gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional

means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (*see, e.g.,* U.S. Patent Nos. 4,683,195 and 4,683,202), such

5   as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see, e.g.,* Landegran, *et al.,* 1988. *Science* 241: 1077-1080; and Nakazawa, *et al.,* 1994. *Proc. Natl. Acad. Sci. USA* 91: 360-364), the latter of which can be particularly useful for detecting point mutations in the SECX and/or NOVX-gene (*see,* Abravaya, *et al.,* 1995. *Nucl. Acids Res.* 23: 675-682). This method can include the steps of collecting a sample of cells from a patient,

10   isolating nucleic acid (*e.g.,* genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to an SECX and/or NOVX gene under conditions such that hybridization and amplification of the SECX and/or NOVX gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the

15   length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (*see,* Guatelli, *et al.,* 1990. *Proc. Natl. Acad. Sci. USA* 87: 1874-1878), transcriptional amplification

20   system (*see,* Kwoh, *et al.,* 1989. *Proc. Natl. Acad. Sci. USA* 86: 1173-1177); Qβ Replicase (*see,* Lizardi, *et al,* 1988. *BioTechnology* 6: 1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

25   In an alternative embodiment, mutations in an SECX and/or NOVX gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and

30   control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (*see, e.g.,* U.S. Patent No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in SECX and/or NOVX can be identified by hybridizing a sample and control nucleic acids, *e.g.,* DNA or RNA, to high-density arrays

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containing hundreds or thousands of oligonucleotides probes. *See, e.g.,* Cronin, *et al.,* 1996. *Human Mutation* 7: 244-255; Kozal, *et al.,* 1996. *Nat. Med.* 2: 753-759. For example, genetic mutations in SECX and/or NOVX can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, *et al., supra.* Briefly, a first hybridization

5   array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each

10   mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the SECX and/or NOVX gene and detect mutations by comparing the sequence of the sample SECX and/or NOVX with the corresponding wild-type

15   (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert, 1977. *Proc. Natl. Acad. Sci. USA* 74: 560 or Sanger, 1977. *Proc. Natl. Acad. Sci. USA* 74: 5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (*see, e.g.,* Naeve, *et al.,* 1995. *Biotechniques* 19: 448), including sequencing by mass spectrometry

20   (see, *e.g.,* PCT International Publication No. WO 94/16101; Cohen, *et al.,* 1996. *Adv. Chromatography* 36: 127-162; and Griffin, *et al.,* 1993. *Appl. Biochem. Biotechnol.* 38: 147-159).

Other methods for detecting mutations in the SECX and/or NOVX gene include methods in which protection from cleavage agents is used to detect mismatched bases in

25   RNA/RNA or RNA/DNA heteroduplexes. *See, e.g.,* Myers, *et al.,* 1985. *Science* 230: 1242. In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type SECX and/or NOVX sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the

30   duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S_1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion

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of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. *See, e.g.,* Cotton, *et al.,* 1988. *Proc. Natl. Acad. Sci. USA* 85: 4397; Saleeba, *et al.,* 1992. *Methods Enzymol.* 217: 286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

5   In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in SECX and/or NOVX cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells

10   cleaves T at G/T mismatches. *See, e.g.,* Hsu, *et al.,* 1994. *Carcinogenesis* 15: 1657-1662. According to an exemplary embodiment, a probe based on an SECX and/or NOVX sequence, *e.g.,* a wild-type SECX and/or NOVX sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. *See,*

15   *e.g.,* U.S. Patent No. 5,459,039.

          In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in SECX and/or NOVX genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids. *See, e.g.,* Orita, *et al.,* 1989. *Proc. Natl. Acad. Sci. USA:*

20   86: 2766; Cotton, 1993. *Mutat. Res.* 285: 125-144; Hayashi, 1992. *Genet. Anal. Tech. Appl.* 9: 73-79. Single-stranded DNA fragments of sample and control SECX and/or NOVX nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or

25   detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. *See, e.g.,* Keen, *et al.,* 1991. *Trends Genet.* 7: 5.

30   In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). *See, e.g.,* Myers, *et al.,* 1985. *Nature* 313: 495. When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich

DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. *See, e.g.,* Rosenbaum and Reissner, 1987. *Biophys. Chem.* 265: 12753.

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. *See, e.g.,* Saiki, *et al.,* 1986. *Nature* 324: 163; Saiki, *et al.,* 1989. *Proc. Natl. Acad. Sci. USA* 86: 6230. Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization; *see, e.g.,* Gibbs, *et al.,* 1989. *Nucl. Acids Res.* 17: 2437-2448) or at the extreme 3'-terminus of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (*see, e.g.,* Prossner, 1993. *Tibtech.* 11: 238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection. *See, e.g.,* Gasparini, *et al.,* 1992. *Mol. Cell Probes* 6: 1. It is anticipated that in certain embodiments amplification may also be performed using *Taq* ligase for amplification. *See, e.g.,* Barany, 1991. *Proc. Natl. Acad. Sci. USA* 88: 189. In such cases, ligation will occur only if there is a perfect match at the 3'-terminus of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.,* in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving an SECX and/or NOVX gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which SECX and/or NOVX is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on SECX and/or NOVX activity (*e.g.*, SECX and/or NOVX gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (The disorders include metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, and hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.) In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.*, drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of SECX and/or NOVX protein, expression of SECX and/or NOVX nucleic acid, or mutation content of SECX and/or NOVX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, 1996. *Clin. Exp. Pharmacol. Physiol.*, 23: 983-985; Linder, 1997. *Clin. Chem.*, 43: 254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is hemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic

polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome PREGNANCY ZONE PROTEIN PRECURSOR enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard

5   and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite

10   frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. At the other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified

15   to be due to CYP2D6 gene amplification.

Thus, the activity of SECX and/or NOVX protein, expression of SECX and/or NOVX nucleic acid, or mutation content of SECX and/or NOVX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of

20   polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with an SECX and/or NOVX modulator, such as a modulator identified by one of the exemplary screening assays described herein.

25

Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of SECX and/or NOVX (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For

30   example, the effectiveness of an agent determined by a screening assay as described herein to increase SECX and/or NOVX gene expression, protein levels, or upregulate SECX and/or NOVX activity, can be monitored in clinical trails of subjects exhibiting decreased SECX and/or NOVX gene expression, protein levels, or downregulated SECX and/or NOVX activity. Alternatively, the effectiveness of an agent determined by a screening assay to

decrease SECX and/or NOVX gene expression, protein levels, or downregulate SECX and/or NOVX activity, can be monitored in clinical trails of subjects exhibiting increased SECX and/or NOVX gene expression, protein levels, or upregulated SECX and/or NOVX activity. In such clinical trials, the expression or activity of SECX and/or NOVX and, preferably, other
5 genes that have been implicated in, for example, a cellular proliferation or immune disorder can be used as a "read out" or markers of the immune responsiveness of a particular cell.

By way of example, and not of limitation, genes, including SECX and/or NOVX, that are modulated in cells by treatment with an agent (e.g., compound, drug or small molecule) that modulates SECX and/or NOVX activity (e.g., identified in a screening assay as described
10 herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of SECX and/or NOVX and other genes implicated in the disorder. The levels of gene expression (i.e., a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein
15 produced, by one of the methods as described herein, or by measuring the levels of activity of SECX and/or NOVX or other genes. In this manner, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

20 In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, protein, peptide, peptidomimetic, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression
25 of an SECX and/or NOVX protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the SECX and/or NOVX protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the SECX and/or NOVX protein, mRNA, or genomic DNA in the pre-administration sample with
30 the SECX and/or NOVX protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of SECX and/or NOVX to higher levels than detected, i.e., to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be

desirable to decrease expression or activity of SECX and/or NOVX to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

Methods of Treatment

The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant SECX and/or NOVX expression or activity. The disorders include cardiomyopathy, atherosclerosis, hypertension, congenital heart defects, aortic stenosis, atrial septal defect (ASD), atrioventricular (A-V) canal defect, ductus arteriosus, pulmonary stenosis, subaortic stenosis, ventricular septal defect (VSD), valve diseases, tuberous sclerosis, scleroderma, obesity, transplantation, adrenoleukodystrophy, congenital adrenal hyperplasia, prostate cancer, neoplasm; adenocarcinoma, lymphoma, uterus cancer, fertility, hemophilia, hypercoagulation, idiopathic thrombocytopenic purpura, immunodeficiencies, graft versus host disease, AIDS, bronchial asthma, Crohn's disease; multiple sclerosis, treatment of Albright Hereditary Ostoeodystrophy, and other diseases, disorders and conditions of the like.

These methods of treatment will be discussed more fully, below.

Disease and Disorders

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to: (*i*) an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; (*ii*) antibodies to an aforementioned peptide; (*iii*) nucleic acids encoding an aforementioned peptide; (*iv*) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to an aforementioned peptide) that are utilized to "knockout" endogenous function of an aforementioned peptide by homologous recombination (*see, e.g.*, Capecchi, 1989. *Science* 244: 1288-1292); or (*v*) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between an aforementioned peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with

Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, an aforementioned peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

5   Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of an aforementioned peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by

10   sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, and the like).

Prophylactic Methods

15   In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant SECX and/or NOVX expression or activity, by administering to the subject an agent that modulates SECX and/or NOVX expression or at least one SECX and/or NOVX activity. Subjects at risk for a disease that is caused or contributed to by aberrant SECX and/or NOVX expression or activity can be identified by, for

20   example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the SECX and/or NOVX aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending upon the type of SECX and/or NOVX aberrancy, for example, an SECX and/or NOVX agonist or SECX and/or

25   NOVX antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein. The prophylactic methods of the invention are further discussed in the following subsections.

Therapeutic Methods

30   Another aspect of the invention pertains to methods of modulating SECX and/or NOVX expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of SECX and/or NOVX protein activity associated with the cell. An agent that modulates SECX and/or NOVX protein activity can be an agent as described herein, such as a nucleic

acid or a protein, a naturally-occurring cognate ligand of an SECX and/or NOVX protein, a
peptide, an SECX and/or NOVX peptidomimetic, or other small molecule. In one
embodiment, the agent stimulates one or more SECX and/or NOVX protein activity.
Examples of such stimulatory agents include active SECX and/or NOVX protein and a nucleic

5   acid molecule encoding SECX and/or NOVX that has been introduced into the cell. In
another embodiment, the agent inhibits one or more SECX and/or NOVX protein activity.
Examples of such inhibitory agents include antisense SECX and/or NOVX nucleic acid
molecules and anti-SECX and/or NOVX antibodies. These modulatory methods can be
performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by

10   administering the agent to a subject). As such, the invention provides methods of treating an
individual afflicted with a disease or disorder characterized by aberrant expression or activity
of an SECX and/or NOVX protein or nucleic acid molecule. In one embodiment, the method
involves administering an agent (*e.g.*, an agent identified by a screening assay described
herein), or combination of agents that modulates (*e.g.*, up-regulates or down-regulates) SECX

15   and/or NOVX expression or activity. In another embodiment, the method involves
administering an SECX and/or NOVX protein or nucleic acid molecule as therapy to
compensate for reduced or aberrant SECX and/or NOVX expression or activity.

Stimulation of SECX and/or NOVX activity is desirable *in situ*ations in which SECX
and/or NOVX is abnormally downregulated and/or in which increased SECX and/or NOVX

20   activity is likely to have a beneficial effect. One example of such a situation is where a subject
has a disorder characterized by aberrant cell proliferation and/or differentiation (*e.g.*, cancer or
immune associated disorders). Another example of such a situation is where the subject has a
gestational disease (*e.g.*, preclampsia).

Determination of the Biological Effect of the Therapeutic

25   In various embodiments of the invention, suitable *in vitro* or *in vivo* assays are
performed to determine the effect of a specific Therapeutic and whether its administration is
indicated for treatment of the affected tissue.

In various specific embodiments, *in vitro* assays may be performed with representative
cells of the type(s) involved in the patient's disorder, to determine if a given Therapeutic exerts

30   the desired effect upon the cell type(s). Compounds for use in therapy may be tested in
suitable animal model systems including, but not limited to rats, mice, chicken, cows,
monkeys, rabbits, and the like, prior to testing in human subjects. Similarly, for *in vivo*

testing, any of the animal model system known in the art may be used prior to administration to human subjects.

Prophylactic and Therapeutic Uses of the Compositions of the Invention

The SECX and/or NOVX nucleic acids and proteins of the invention are useful in potential prophylactic and therapeutic applications implicated in a variety of disorders including, but not limited to: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias, metabolic disturbances associated with obesity, the metabolic syndrome X and wasting disorders associated with chronic diseases and various cancers.

As an example, a cDNA encoding the SECX and/or NOVX protein of the invention may be useful in gene therapy, and the protein may be useful when administered to a subject in need thereof. By way of non-limiting example, the compositions of the invention will have efficacy for treatment of patients suffering from: metabolic disorders, diabetes, obesity, infectious disease, anorexia, cancer-associated cachexia, cancer, neurodegenerative disorders, Alzheimer's Disease, Parkinson's Disorder, immune disorders, hematopoietic disorders, and the various dyslipidemias.

Both the novel nucleic acid encoding the SECX and/or NOVX protein, and the SECX and/or NOVX protein of the invention, or fragments thereof, may also be useful in diagnostic applications, wherein the presence or amount of the nucleic acid or the protein are to be assessed. A further use could be as an anti-bacterial molecule (*i.e.,* some peptides have been found to possess anti-bacterial properties). These materials are further useful in the generation of antibodies, which immunospecifically-bind to the novel substances of the invention for use in therapeutic or diagnostic methods.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

Examples

Example 1. Identification of SECX and/or NOVX clones

The novel SECX and/or NOVX target sequences identified in the present invention were subjected to the exon linking process to confirm the sequence. PCR primers were designed by starting at the most upstream sequence available, for the forward primer, and at the most downstream sequence available for the reverse primer. Table 15A shows the

sequences of the PCR primers used for obtaining different clones. In each case, the sequence was examined, walking inward from the respective termini toward the coding sequence, until a suitable sequence that is either unique or highly selective was encountered, or, in the case of the reverse primer, until the stop codon was reached. Such primers were designed based on in

5   silico predictions for the full length cDNA, part (one or more exons) of the DNA or protein sequence of the target sequence, or by translated homology of the predicted exons to closely related human sequences from other species. These primers were then employed in PCR amplification based on the following pool of human cDNAs: adrenal gland, bone marrow, brain - amygdala, brain - cerebellum, brain - hippocampus, brain - substantia nigra, brain -

10   thalamus, brain -whole, fetal brain, fetal kidney, fetal liver, fetal lung, heart, kidney, lymphoma - Raji, mammary gland, pancreas, pituitary gland, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thyroid, trachea, uterus. Usually the resulting amplicons were gel purified, cloned and sequenced to high redundancy. The PCR product derived from exon linking was cloned into the pCR2.1 vector

15   from Invitrogen. The resulting bacterial clone has an insert covering the entire open reading frame cloned into the pCR2.1 vector. The resulting sequences from all clones were assembled with themselves, with other fragments in CuraGen Corporation's database and with public ESTs. Fragments and ESTs were included as components for an assembly when the extent of their identity with another component of the assembly was at least 95% over 50 bp. In

20   addition, sequence traces were evaluated manually and edited for corrections if appropriate. These procedures provide the sequence reported herein.

Physical clone: Exons were predicted by homology and the intron/exon boundaries were determined using standard genetic rules. Exons were further selected and refined by means of similarity determination using multiple BLAST (for example, tBlastN, BlastX, and

25   BlastN) searches, and, in some instances, GeneScan and Grail. Expressed sequences from both public and proprietary databases were also added when available to further define and complete the gene sequence. The DNA sequence was then manually corrected for apparent inconsistencies thereby obtaining the sequences encoding the full-length protein.

30   **Example 2. Quantitative expression analysis of clones in various cells and tissues**

The quantitative expression of various clones was assessed using microtiter plates containing RNA samples from a variety of normal and pathology-derived cells, cell lines and tissues using real time quantitative PCR (RTQ PCR). RTQ PCR was performed on an Applied Biosystems ABI PRISM® 7700 or an ABI PRISM® 7900 HT Sequence Detection System.

Various collections of samples are assembled on the plates, and referred to as Panel 1 (containing normal tissues and cancer cell lines), Panel 2 (containing samples derived from tissues from normal and cancer sources), Panel 3 (containing cancer cell lines), Panel 4 (containing cells and cell lines from normal tissues and cells related to inflammatory

5   conditions), Panel 5D/5I (containing human tissues and cell lines with an emphasis on metabolic diseases), AI_comprehensive_panel (containing normal tissue and samples from autoimmune diseases), Panel CNSD.01 (containing central nervous system samples from normal and diseased brains) and CNS_neurodegeneration_panel (containing samples from normal and Alzheimer's diseased brains).

10   RNA integrity from all samples is controlled for quality by visual assessment of agarose gel electropherograms using 28S and 18S ribosomal RNA staining intensity ratio as a guide (2:1 to 2.5:1 28s:18s) and the absence of low molecular weight RNAs that would be indicative of degradation products. Samples are controlled against genomic DNA contamination by RTQ PCR reactions run in the absence of reverse transcriptase using probe

15   and primer sets designed to amplify across the span of a single exon.

First, the RNA samples were normalized to reference nucleic acids such as constitutively expressed genes (for example, β-actin and GAPDH). Normalized RNA (5 ul) was converted to cDNA and analyzed by RTQ-PCR using One Step RT-PCR Master Mix Reagents (Applied Biosystems; Catalog No. 4309169) and gene-specific primers according to

20   the manufacturer's instructions.

In other cases, non-normalized RNA samples were converted to single strand cDNA (sscDNA) using Superscript II (Invitrogen Corporation; Catalog No. 18064-147) and random hexamers according to the manufacturer's instructions. Reactions containing up to 10 µg of total RNA were performed in a volume of 20 µl and incubated for 60 minutes at 42°C. This

25   reaction can be scaled up to 50 µg of total RNA in a final volume of 100 µl. sscDNA samples are then normalized to reference nucleic acids as described previously, using 1X TaqMan® Universal Master mix (Applied Biosystems; catalog No. 4324020), following the manufacturer's instructions.

Probes and primers were designed for each assay according to Applied Biosystems

30   Primer Express Software package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration = 250 nM, primer melting temperature (Tm) range = 58°-60°C, primer optimal

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Tm = 59°C, maximum primer difference = 2°C, probe does not have 5'G, probe Tm must be 10°C greater than primer Tm, amplicon size 75bp to 100bp. The probes and primers selected (see below) were synthesized by Synthegen (Houston, TX, USA). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of

5   reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900nM each, and probe, 200nM.

PCR conditions: When working with RNA samples, normalized RNA from each tissue and each cell line was spotted in each well of either a 96 well or a 384-well PCR plate (Applied Biosystems). PCR cocktails included either a single gene specific probe and primers

10   set, or two multiplexed probe and primers sets (a set specific for the target clone and another gene-specific set multiplexed with the target probe). PCR reactions were set up using TaqMan® One-Step RT-PCR Master Mix (Applied Biosystems, Catalog No. 4313803) following manufacturer's instructions. Reverse transcription was performed at 48°C for 30 minutes followed by amplification/PCR cycles as follows: 95°C 10 min, then 40 cycles of

15   95°C for 15 seconds, 60°C for 1 minute. Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100.

20   When working with sscDNA samples, normalized sscDNA was used as described previously for RNA samples. PCR reactions containing one or two sets of probe and primers were set up as described previously, using 1X TaqMan® Universal Master mix (Applied Biosystems; catalog No. 4324020), following the manufacturer's instructions. PCR amplification was performed as follows: 95°C 10 min, then 40 cycles of 95°C for 15 seconds,

25   60°C for 1 minute. Results were analyzed and processed as described previously.

Panels 1, 1.1, 1.2, and 1.3D

The plates for Panels 1, 1.1, 1.2 and 1.3D include 2 control wells (genomic DNA control and chemistry control) and 94 wells containing cDNA from various samples. The samples in these panels are broken into 2 classes: samples derived from cultured cell lines and

30   samples derived from primary normal tissues. The cell lines are derived from cancers of the following types: lung cancer, breast cancer, melanoma, colon cancer, prostate cancer, CNS cancer, squamous cell carcinoma, ovarian cancer, liver cancer, renal cancer, gastric cancer and pancreatic cancer. Cell lines used in these panels are widely available through the American

Type Culture Collection (ATCC), a repository for cultured cell lines, and were cultured using the conditions recommended by the ATCC. The normal tissues found on these panels are comprised of samples derived from all major organ systems from single adult individuals or fetuses. These samples are derived from the following organs: adult skeletal muscle, fetal

5   skeletal muscle, adult heart, fetal heart, adult kidney, fetal kidney, adult liver, fetal liver, adult lung, fetal lung, various regions of the brain, the spleen, bone marrow, lymph node, pancreas, salivary gland, pituitary gland, adrenal gland, spinal cord, thymus, stomach, small intestine, colon, bladder, trachea, breast, ovary, uterus, placenta, prostate, testis and adipose.

In the results for Panels 1, 1.1, 1.2 and 1.3D, the following abbreviations are used:

10   ca. = carcinoma,

'   * = established from metastasis,

met = metastasis,

s cell var = small cell variant,

non-s = non-sm = non-small,

15   squam = squamous,

pl. eff = pl effusion = pleural effusion,

glio = glioma,

astro = astrocytoma, and

neuro = neuroblastoma.

20   **General_screening_panel_v1.4**

The plates for Panel 1.4 include 2 control wells (genomic DNA control and chemistry control) and 94 wells containing cDNA from various samples. The samples in Panel 1.4 are broken into 2 classes: samples derived from cultured cell lines and samples derived from primary normal tissues. The cell lines are derived from cancers of the following types: lung

25   cancer, breast cancer, melanoma, colon cancer, prostate cancer, CNS cancer, squamous cell carcinoma, ovarian cancer, liver cancer, renal cancer, gastric cancer and pancreatic cancer. Cell lines used in Panel 1.4 are widely available through the American Type Culture Collection (ATCC), a repository for cultured cell lines, and were cultured using the conditions recommended by the ATCC. The normal tissues found on Panel 1.4 are comprised of pools of

30   samples derived from all major organ systems from 2 to 5 different adult individuals or fetuses. These samples are derived from the following organs: adult skeletal muscle, fetal skeletal muscle, adult heart, fetal heart, adult kidney, fetal kidney, adult liver, fetal liver, adult lung, fetal lung, various regions of the brain, the spleen, bone marrow, lymph node, pancreas,

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salivary gland, pituitary gland, adrenal gland, spinal cord, thymus, stomach, small intestine, colon, bladder, trachea, breast, ovary, uterus, placenta, prostate, testis and adipose. Abbreviations are as described for Panels 1, 1.1, 1.2, and 1.3D.

Panels 2D and 2.2

5   The plates for Panels 2D and 2.2 generally include 2 control wells and 94 test samples composed of RNA or cDNA isolated from human tissue procured by surgeons working in close cooperation with the National Cancer Institute's Cooperative Human Tissue Network (CHTN) or the National Disease Research Initiative (NDRI). The tissues are derived from human malignancies and in cases where indicated many malignant tissues have "matched

10   margins" obtained from noncancerous tissue just adjacent to the tumor. These are termed normal adjacent tissues and are denoted "NAT" in the results below. The tumor tissue and the "matched margins" are evaluated by two independent pathologists (the surgical pathologists and again by a pathologist at NDRI or CHTN). This analysis provides a gross histopathological assessment of tumor differentiation grade. Moreover, most samples include

15   the original surgical pathology report that provides information regarding the clinical stage of the patient. These matched margins are taken from the tissue surrounding (i.e. immediately proximal) to the zone of surgery (designated "NAT", for normal adjacent tissue, in Table RR). In addition, RNA and cDNA samples were obtained from various human tissues derived from autopsies performed on elderly people or sudden death victims (accidents, etc.). These tissues

20   were ascertained to be free of disease and were purchased from various commercial sources such as Clontech (Palo Alto, CA), Research Genetics, and Invitrogen.

Panel 3D

The plates of Panel 3D are comprised of 94 cDNA samples and two control samples. Specifically, 92 of these samples are derived from cultured human cancer cell lines, 2 samples

25   of human primary cerebellar tissue and 2 controls. The human cell lines are generally obtained from ATCC (American Type Culture Collection), NCI or the German tumor cell bank and fall into the following tissue groups: Squamous cell carcinoma of the tongue, breast cancer, prostate cancer, melanoma, epidermoid carcinoma, sarcomas, bladder carcinomas, pancreatic cancers, kidney cancers, leukemias/lymphomas, ovarian/uterine/cervical, gastric, colon, lung

30   and CNS cancer cell lines. In addition, there are two independent samples of cerebellum. These cells are all cultured under standard recommended conditions and RNA extracted using the standard procedures. The cell lines in panel 3D and 1.3D are of the most common cell lines used in the scientific literature.

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Panels 4D, 4R, and 4.1D

Panel 4 includes samples on a 96 well plate (2 control wells, 94 test samples) composed of RNA (Panel 4R) or cDNA (Panels 4D/4.1D) isolated from various human cell lines or tissues related to inflammatory conditions. Total RNA from control normal tissues such as colon and lung (Stratagene, La Jolla, CA) and thymus and kidney (Clontech) was employed. Total RNA from liver tissue from cirrhosis patients and kidney from lupus patients was obtained from BioChain (Biochain Institute, Inc., Hayward, CA). Intestinal tissue for RNA preparation from patients diagnosed as having Crohn's disease and ulcerative colitis was obtained from the National Disease Research Interchange (NDRI) (Philadelphia, PA).

Astrocytes, lung fibroblasts, dermal fibroblasts, coronary artery smooth muscle cells, small airway epithelium, bronchial epithelium, microvascular dermal endothelial cells, microvascular lung endothelial cells, human pulmonary aortic endothelial cells, human umbilical vein endothelial cells were all purchased from Clonetics (Walkersville, MD) and grown in the media supplied for these cell types by Clonetics. These primary cell types were activated with various cytokines or combinations of cytokines for 6 and/or 12-14 hours, as indicated. The following cytokines were used; IL-1 beta at approximately 1-5ng/ml, TNF alpha at approximately 5-10ng/ml, IFN gamma at approximately 20-50ng/ml, IL-4 at approximately 5-10ng/ml, IL-9 at approximately 5-10ng/ml, IL-13 at approximately 5-10ng/ml. Endothelial cells were sometimes starved for various times by culture in the basal media from Clonetics with 0.1% serum.

Mononuclear cells were prepared from blood of employees at CuraGen Corporation, using Ficoll. LAK cells were prepared from these cells by culture in DMEM 5% FCS (Hyclone), 100μM non essential amino acids (Gibco/Life Technologies, Rockville, MD), 1mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5}M (Gibco), and 10mM Hepes (Gibco) and Interleukin 2 for 4-6 days. Cells were then either activated with 10-20ng/ml PMA and 1-2μg/ml ionomycin, IL-12 at 5-10ng/ml, IFN gamma at 20-50ng/ml and IL-18 at 5-10ng/ml for 6 hours. In some cases, mononuclear cells were cultured for 4-5 days in DMEM 5% FCS (Hyclone), 100μM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5}M (Gibco), and 10mM Hepes (Gibco) with PHA (phytohemagglutinin) or PWM (pokeweed mitogen) at approximately 5μg/ml. Samples were taken at 24, 48 and 72 hours for RNA preparation. MLR (mixed lymphocyte reaction) samples were obtained by taking blood from two donors, isolating the mononuclear cells using Ficoll and mixing the isolated mononuclear cells 1:1 at a final concentration of approximately 2×10^6cells/ml in DMEM 5% FCS (Hyclone), 100μM non essential amino acids (Gibco), 1mM

sodium pyruvate (Gibco), mercaptoethanol (5.5×10^{-5}M) (Gibco), and 10mM Hepes (Gibco). The MLR was cultured and samples taken at various time points ranging from 1- 7 days for RNA preparation.

5　　　Monocytes were isolated from mononuclear cells using CD14 Miltenyi Beads, +ve VS selection columns and a Vario Magnet according to the manufacturer's instructions. Monocytes were differentiated into dendritic cells by culture in DMEM 5% fetal calf serum (FCS) (Hyclone, Logan, UT), 100μM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5}M (Gibco), and 10mM Hepes (Gibco), 50ng/ml GMCSF and 5ng/ml IL-4 for 5-7 days. Macrophages were prepared by culture of monocytes

10　　for 5-7 days in DMEM 5% FCS (Hyclone), 100μM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5}M (Gibco), 10mM Hepes (Gibco) and 10% AB Human Serum or MCSF at approximately 50ng/ml. Monocytes, macrophages and dendritic cells were stimulated for 6 and 12-14 hours with lipopolysaccharide (LPS) at 100ng/ml. Dendritic cells were also stimulated with anti-CD40 monoclonal antibody

15　　(Pharmingen) at 10μg/ml for 6 and 12-14 hours.

　　　　CD4 lymphocytes, CD8 lymphocytes and NK cells were also isolated from mononuclear cells using CD4, CD8 and CD56 Miltenyi beads, positive VS selection columns and a Vario Magnet according to the manufacturer's instructions. CD45RA and CD45RO CD4 lymphocytes were isolated by depleting mononuclear cells of CD8, CD56, CD14 and CD19

20　　cells using CD8, CD56, CD14 and CD19 Miltenyi beads and positive selection. CD45RO beads were then used to isolate the CD45RO CD4 lymphocytes with the remaining cells being CD45RA CD4 lymphocytes. CD45RA CD4, CD45RO CD4 and CD8 lymphocytes were placed in DMEM 5% FCS (Hyclone), 100μM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5}M (Gibco), and 10mM Hepes (Gibco) and

25　　plated at 10^6cells/ml onto Falcon 6 well tissue culture plates that had been coated overnight with 0.5μg/ml anti-CD28 (Pharmingen) and 3ug/ml anti-CD3 (OKT3, ATCC) in PBS. After 6 and 24 hours, the cells were harvested for RNA preparation. To prepare chronically activated CD8 lymphocytes, we activated the isolated CD8 lymphocytes for 4 days on anti-CD28 and anti-CD3 coated plates and then harvested the cells and expanded them in DMEM 5% FCS

30　　(Hyclone), 100μM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol 5.5×10^{-5}M (Gibco), and 10mM Hepes (Gibco) and IL-2. The expanded CD8 cells were then activated again with plate bound anti-CD3 and anti-CD28 for 4 days and expanded as before. RNA was isolated 6 and 24 hours after the second activation and after 4 days of the second expansion culture. The isolated NK cells were cultured in DMEM 5% FCS

(Hyclone), 100μM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol $5.5x10^{-5}M$ (Gibco), and 10mM Hepes (Gibco) and IL-2 for 4-6 days before RNA was prepared.

To obtain B cells, tonsils were procured from NDRI. The tonsil was cut up with sterile
5   dissecting scissors and then passed through a sieve. Tonsil cells were then spun down and resupended at 10^{6}cells/ml in DMEM 5% FCS (Hyclone), 100μM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol $5.5x10^{-5}M$ (Gibco), and 10mM Hepes (Gibco). To activate the cells, we used PWM at 5μg/ml or anti-CD40 (Pharmingen) at approximately 10μg/ml and IL-4 at 5-10ng/ml. Cells were harvested for RNA preparation at
10 24,48 and 72 hours.

To prepare the primary and secondary Th1/Th2 and Tr1 cells, six-well Falcon plates were coated overnight with 10μg/ml anti-CD28 (Pharmingen) and 2μg/ml OKT3 (ATCC), and then washed twice with PBS. Umbilical cord blood CD4 lymphocytes (Poietic Systems, German Town, MD) were cultured at 10^{5}-10^{6}cells/ml in DMEM 5% FCS (Hyclone), 100μM
15 non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol $5.5x10^{-5}M$ (Gibco), 10mM Hepes (Gibco) and IL-2 (4ng/ml). IL-12 (5ng/ml) and anti-IL4 (1μg/ml) were used to direct to Th1, while IL-4 (5ng/ml) and anti-IFN gamma (1μg/ml) were used to direct to Th2 and IL-10 at 5ng/ml was used to direct to Tr1. After 4-5 days, the activated Th1, Th2 and Tr1 lymphocytes were washed once in DMEM and expanded for 4-7 days in DMEM
20 5% FCS (Hyclone), 100μM non essential amino acids (Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol $5.5x10^{-5}M$ (Gibco), 10mM Hepes (Gibco) and IL-2 (1ng/ml). Following this, the activated Th1, Th2 and Tr1 lymphocytes were re-stimulated for 5 days with anti-CD28/OKT3 and cytokines as described above, but with the addition of anti-CD95L (1μg/ml) to prevent apoptosis. After 4-5 days, the Th1, Th2 and Tr1 lymphocytes were
25 washed and then expanded again with IL-2 for 4-7 days. Activated Th1 and Th2 lymphocytes were maintained in this way for a maximum of three cycles. RNA was prepared from primary and secondary Th1, Th2 and Tr1 after 6 and 24 hours following the second and third activations with plate bound anti-CD3 and anti-CD28 mAbs and 4 days into the second and third expansion cultures in Interleukin 2.

30 The following leukocyte cells lines were obtained from the ATCC: Ramos, EOL-1, KU-812. EOL cells were further differentiated by culture in 0.1mM dbcAMP at $5x10^{5}$cells/ml for 8 days, changing the media every 3 days and adjusting the cell concentration to $5x10^{5}$cells/ml. For the culture of these cells, we used DMEM or RPMI (as recommended by the ATCC), with the addition of 5% FCS (Hyclone), 100μM non essential amino acids

(Gibco), 1mM sodium pyruvate (Gibco), mercaptoethanol 5.5x10⁻³M (Gibco), 10mM Hepes

(Gibco). RNA was either prepared from resting cells or cells activated with PMA at 10ng/ml

and ionomycin at 1µg/ml for 6 and 14 hours. Keratinocyte line CCD106 and an airway

epithelial tumor line NCI-H292 were also obtained from the ATCC. Both were cultured in

5   DMEM 5% FCS (Hyclone), 100µM non essential amino acids (Gibco), 1mM sodium pyruvate

(Gibco), mercaptoethanol 5.5x10⁻⁵M (Gibco), and 10mM Hepes (Gibco). CCD1106 cells were

activated for 6 and 14 hours with approximately 5 ng/ml TNF alpha and 1ng/ml IL-1 beta,

while NCI-H292 cells were activated for 6 and 14 hours with the following cytokines: 5ng/ml

IL-4, 5ng/ml IL-9, 5ng/ml IL-13 and 25ng/ml IFN gamma.

10   For these cell lines and blood cells, RNA was prepared by lysing approximately

10⁷cells/ml using Trizol (Gibco BRL). Briefly, 1/10 volume of bromochloropropane

(Molecular Research Corporation) was added to the RNA sample, vortexed and after 10

minutes at room temperature, the tubes were spun at 14,000 rpm in a Sorvall SS34 rotor. The

aqueous phase was removed and placed in a 15ml Falcon Tube. An equal volume of

15   isopropanol was added and left at -20°C overnight. The precipitated RNA was spun down at

9,000 rpm for 15 min in a Sorvall SS34 rotor and washed in 70% ethanol. The pellet was

redissolved in 300µl of RNAse-free water and 35µl buffer (Promega) 5µl DTT, 7µl RNAsin

and 8µl DNAse were added. The tube was incubated at 37°C for 30 minutes to remove

contaminating genomic DNA, extracted once with phenol chloroform and re-precipitated with

20   1/10 volume of 3M sodium acetate and 2 volumes of 100% ethanol. The RNA was spun down

and placed in RNAse free water. RNA was stored at -80°C.

AI_comprehensive panel_v1.0

The plates for AI_comprehensive panel_v1.0 include two control wells and 89 test

samples comprised of cDNA isolated from surgical and postmortem human tissues obtained

25   from the Backus Hospital and Clinomics (Frederick, MD). Total RNA was extracted from

tissue samples from the Backus Hospital in the Facility at CuraGen. Total RNA from other

tissues was obtained from Clinomics.

Joint tissues including synovial fluid, synovium, bone and cartilage were obtained from

patients undergoing total knee or hip replacement surgery at the Backus Hospital. Tissue

30   samples were immediately snap frozen in liquid nitrogen to ensure that isolated RNA was of

optimal quality and not degraded. Additional samples of osteoarthritis and rheumatoid arthritis

joint tissues were obtained from Clinomics. Normal control tissues were supplied by

Clinomics and were obtained during autopsy of trauma victims.

Surgical specimens of psoriatic tissues and adjacent matched tissues were provided as total RNA by Clinomics. Two male and two female patients were selected between the ages of 25 and 47. None of the patients were taking prescription drugs at the time samples were isolated.

5   Surgical specimens of diseased colon from patients with ulcerative colitis and Crohns disease and adjacent matched tissues were obtained from Clinomics. Bowel tissue from three female and three male Crohn's patients between the ages of 41-69 were used. Two patients were not on prescription medication while the others were taking dexamethasone, phenobarbital, or tylenol. Ulcerative colitis tissue was from three male and four female

10   patients. Four of the patients were taking lebvid and two were on phenobarbital.

Total RNA from post mortem lung tissue from trauma victims with no disease or with emphysema, asthma or COPD was purchased from Clinomics. Emphysema patients ranged in age from 40-70 and all were smokers, this age range was chosen to focus on patients with cigarette-linked emphysema and to avoid those patients with alpha-1anti-trypsin deficiencies.

15   Asthma patients ranged in age from 36-75, and excluded smokers to prevent those patients that could also have COPD. COPD patients ranged in age from 35-80 and included both smokers and non-smokers. Most patients were taking corticosteroids, and bronchodilators.

In the labels employed to identify tissues in the AI_comprehensive panel_v1.0 panel, the following abbreviations are used:

20   AI = Autoimmunity

Syn = Synovial

Normal = No apparent disease

Rep22 /Rep20 = individual patients

RA = Rheumatoid arthritis

25   Backus = From Backus Hospital

OA = Osteoarthritis

(SS) (BA) (MF) = Individual patients

Adj = Adjacent tissue

Match control = adjacent tissues

30   -M = Male

-F = Female

COPD = Chronic obstructive pulmonary disease

Panels 5D and 5I

The plates for Panel 5D and 5I include two control wells and a variety of cDNAs isolated from human tissues and cell lines with an emphasis on metabolic diseases. Metabolic tissues were obtained from patients enrolled in the Gestational Diabetes study. Cells were obtained during different stages in the differentiation of adipocytes from human mesenchymal

5   stem cells. Human pancreatic islets were also obtained.

In the Gestational Diabetes study subjects are young (18 - 40 years), otherwise healthy women with and without gestational diabetes undergoing routine (elective) Caesarean section. After delivery of the infant, when the surgical incisions were being repaired/closed, the obstetrician removed a small sample (<1 cc) of the exposed metabolic tissues during the

10   closure of each surgical level. The biopsy material was rinsed in sterile saline, blotted and fast frozen within 5 minutes from the time of removal. The tissue was then flash frozen in liquid nitrogen and stored, individually, in sterile screw-top tubes and kept on dry ice for shipment to or to be picked up by CuraGen.   The metabolic tissues of interest include uterine wall (smooth muscle), visceral adipose, skeletal muscle (rectus) and subcutaneous adipose.   Patient

15   descriptions are as follows:

Patient 2	Diabetic Hispanic, overweight, not on insulin
Patient 7-9	Nondiabetic Caucasian and obese (BMI>30)
Patient 10	Diabetic Hispanic, overweight, on insulin
Patient 11	Nondiabetic African American and overweight
Patient 12	Diabetic Hispanic on insulin

20

Adipocyte differentiation was induced in donor progenitor cells obtained from Osirus (a division of Clonetics/BioWhittaker) in triplicate, except for Donor 3U which had only two

25   replicates. Scientists at Clonetics isolated, grew and differentiated human mesenchymal stem cells (HuMSCs) for CuraGen based on the published protocol found in Mark F. Pittenger, et al., Multilineage Potential of Adult Human Mesenchymal Stem Cells Science Apr 2 1999: 143-147. Clonetics provided Trizol lysates or frozen pellets suitable for mRNA isolation and ds cDNA production. A general description of each donor is as follows:

30   Donor 2 and 3 U: Mesenchymal Stem cells, Undifferentiated Adipose
        Donor 2 and 3 AM: Adipose, AdiposeMidway Differentiated
        Donor 2 and 3 AD: Adipose, Adipose Differentiated

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Human cell lines were generally obtained from ATCC (American Type Culture Collection), NCI or the German tumor cell bank and fall into the following tissue groups: kidney proximal convoluted tubule, uterine smooth muscle cells, small intestine, liver HepG2 cancer cells, heart primary stromal cells, and adrenal cortical adenoma cells. These cells are all cultured under standard recommended conditions and RNA extracted using the standard procedures. All samples were processed at CuraGen to produce single stranded cDNA.

Panel 5I contains all samples previously described with the addition of pancreatic islets from a 58 year old female patient obtained from the Diabetes Research Institute at the University of Miami School of Medicine. Islet tissue was processed to total RNA at an outside source and delivered to CuraGen for addition to panel 5I.

In the labels employed to identify tissues in the 5D and 5I panels, the following abbreviations are used:

GO Adipose = Greater Omentum Adipose

SK = Skeletal Muscle

UT = Uterus

PL = Placenta

AD = Adipose Differentiated

AM = Adipose Midway Differentiated

U = Undifferentiated Stem Cells

Panel CNSD.01

The plates for Panel CNSD.01 include two control wells and 94 test samples comprised of cDNA isolated from postmortem human brain tissue obtained from the Harvard Brain Tissue Resource Center. Brains are removed from calvaria of donors between 4 and 24 hours after death, sectioned by neuroanatomists, and frozen at -80°C in liquid nitrogen vapor. All brains are sectioned and examined by neuropathologists to confirm diagnoses with clear associated neuropathology.

Disease diagnoses are taken from patient records. The panel contains two brains from each of the following diagnoses: Alzheimer's disease, Parkinson's disease, Huntington's disease, Progressive Supernuclear Palsy, Depression, and "Normal controls". Within each of these brains, the following regions are represented: cingulate gyrus, temporal pole, globus palladus, substantia nigra, Brodman Area 4 (primary motor strip), Brodman Area 7 (parietal cortex), Brodman Area 9 (prefrontal cortex), and Brodman area 17 (occipital cortex). Not all brain regions are represented in all cases; e.g., Huntington's disease is characterized in part by

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neurodegeneration in the globus palladus, thus this region is impossible to obtain from confirmed Huntington's cases. Likewise Parkinson's disease is characterized by degeneration of the substantia nigra making this region more difficult to obtain. Normal control brains were examined for neuropathology and found to be free of any pathology consistent with neurodegeneration.

In the labels employed to identify tissues in the CNS panel, the following abbreviations are used:

PSP = Progressive supranuclear palsy

Sub Nigra = Substantia nigra

Glob Palladus= Globus palladus

Temp Pole = Temporal pole

Cing Gyr = Cingulate gyrus

BA 4 = Brodman Area 4

Panel CNS_Neurodegeneration_V1.0

The plates for Panel CNS_Neurodegeneration_V1.0 include two control wells and 47 test samples comprised of cDNA isolated from postmortem human brain tissue obtained from the Harvard Brain Tissue Resource Center (McLean Hospital) and the Human Brain and Spinal Fluid Resource Center (VA Greater Los Angeles Healthcare System). Brains are removed from calvaria of donors between 4 and 24 hours after death, sectioned by neuroanatomists, and frozen at -80°C in liquid nitrogen vapor. All brains are sectioned and examined by neuropathologists to confirm diagnoses with clear associated neuropathology.

Disease diagnoses are taken from patient records. The panel contains six brains from Alzheimer's disease (AD) patients, and eight brains from "Normal controls" who showed no evidence of dementia prior to death. The eight normal control brains are divided into two categories: Controls with no dementia and no Alzheimer's like pathology (Controls) and controls with no dementia but evidence of severe Alzheimer's like pathology; (specifically senile plaque load rated as level 3 on a scale of 0-3; 0 = no evidence of plaques, 3 = severe AD senile plaque load). Within each of these brains, the following regions are represented: hippocampus, temporal cortex (Brodman Area 21), parietal cortex (Brodman area 7), and occipital cortex (Brodman area 17). These regions were chosen to encompass all levels of neurodegeneration in AD. The hippocampus is a region of early and severe neuronal loss in AD; the temporal cortex is known to show neurodegeneration in AD after the hippocampus; the parietal cortex shows moderate neuronal death in the late stages of the disease; the

occipital cortex is spared in AD and therefore acts as a "control" region within AD patients. Not all brain regions are represented in all cases.

In the labels employed to identify tissues in the CNS_Neurodegeneration_V1.0 panel, the following abbreviations are used:

5   AD = Alzheimer's disease brain; patient was demented and showed AD-like pathology upon autopsy

Control = Control brains; patient not demented, showing no neuropathology

Control (Path) = Control brains; pateint not demented but showing sever AD-like pathology

10   SupTemporal Ctx = Superior Temporal Cortex

Inf Temporal Ctx = Inferior Temporal Cortex

SEC11 (CG50379-01)

Expression of gene CG50379-01 was assessed using the primer-probe set Ag2255, described in Table 21A. Results of the RTQ-PCR runs are shown in Tables 21B, 21C, 21D, 21E and 21F.

15   Table 21A. Probe Name Ag2255

Primers	Sequences	Length	Start Position
Forward	5'-tgcaaaatgaacaaccagacta-3' (SEQ ID NO:141)	22	1461
Probe	TET-5'-atccccgccgtggagatcttcat-3'-TAMRA (SEQ ID NO:142)	23	1512
Reverse	5'-ccagcagcataaagatcttcac-3' (SEQ ID NO:143)	22	1536

Table 21B. AI_comprehensive panel_v1.0

Tissue Name	Rel. Exp.(%) Ag2255, Run 228157363	Rel. Exp.(%) Ag2255, Run 228175007	Tissue Name	Rel. Exp.(%) Ag2255, Run 228157363	Rel. Exp.(%) Ag2255, Run 228175007
110967 COPD-F	3.1	4.3	112427 Match Control Psoriasis-F	35.8	28.7
110980 COPD-F	0.0	3.0	112418 Psoriasis-M	5.7	2.6
110968 COPD-M	5.0	5.2	112723 Match Control Psoriasis-M	7.6	3.4
110977 COPD-M	6.0	4.5	112419 Psoriasis-M	9.2	6.3
110989 Emphysema-F	58.6	69.3	112424 Match Control Psoriasis-M	11.7	12.1
110992 Emphysema-F	32.5	29.5	112420 Psoriasis-M	63.3	44.4
110993 Emphysema-F	4.8	4.5	112425 Match Control Psoriasis-M	41.2	31.2
110994 Emphysema-F	0.9	2.1	104689 (MF) OA Bone-Backus	44.1	35.6
110995	100.0	100.0	104690 (MF) Adj	9.1	13.2

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Emphysema-F			"Normal" Bone-Backus		
110996 Emphysema-F	18.7	24.7	104691 (MF) OA Synovium-Backus	88.3	80.7
110997 Asthma-M	15.8	13.7	104692 (BA) OA Cartilage-Backus	37.4	30.1
111001 Asthma-F	17.7	17.8	104694 (BA) OA Bone-Backus	44.1	44.1
111002 Asthma-F	36.6	0.3	104695 (BA) Adj "Normal" Bone-Backus	9.0	9.8
111003 Atopic Asthma-F	34.2	21.0	104696 (BA) OA Synovium-Backus	88.3	98.6
111004 Atopic Asthma-F	42.0	36.6	104700 (SS) OA Bone-Backus	11.0	9.2
111005 Atopic Asthma-F	24.1	26.4	104701 (SS) Adj "Normal" Bone-Backus	44.8	26.8
111006 Atopic Asthma-F	8.2	1.1	104702 (SS) OA Synovium-Backus	56.3	46.0
111417 Allergy-M	33.2	27.7	117093 OA Cartilage Rep7	16.0	21.3
112347 Allergy-M	2.1	0.3	112672 OA Bone5	27.2	10.4
112349 Normal Lung-F	0.9	0.9	112673 OA Synovium5	4.5	0.8
112357 Normal Lung-F	12.7	17.8	112674 OA Synovial Fluid cells5	7.2	8.0
112354 Normal Lung-M	8.0	9.9	117100 OA Cartilage Rep14	8.3	7.2
112374 Crohns-F	22.5	10.5	112756 OA Bone9	82.4	48.6
112389 Match Control Crohns-F	63.7	57.8	112757 OA Synovium9	15.1	9.7
112375 Crohns-F	24.8	18.3	112758 OA Synovial Fluid Cells9	1.1	10.6
112732 Match Control Crohns-F	17.3	30.1	117125 RA Cartilage Rep2	0.0	3.8
112725 Crohns-M	0.8	5.1	113492 Bone2 RA	4.6	6.8
112387 Match Control Crohns-M	32.1	7.6	113493 Synovium2 RA	1.5	2.9
112378 Crohns-M	1.6	0.9	113494 Syn Fluid Cells RA	7.3	4.4
112390 Match Control Crohns-M	73.2	66.9	113499 Cartilage4 RA	2.1	3.4
112726 Crohns-M	36.6	22.7	113500 Bone4 RA	3.0	3.3
112731 Match Control Crohns-M	30.6	24.0	113501 Synovium4 RA	4.3	3.6
112380 Ulcer Col-F	16.3	19.6	113502 Syn Fluid Cells4 RA	2.1	2.5
112734 Match Control Ulcer Col-F	30.8	30.4	113495 Cartilage3 RA	5.7	4.1

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112384 Ulcer Col-F	65.5	71.7	113496 Bone3 RA	2.1	3.0
112737 Match Control Ulcer Col-F	32.3	0.0	113497 Synovium3 RA	2.3	0.0
112386 Ulcer Col-F	2.0	7.1	113498 Syn Fluid Cells3 RA	1.2	6.3
112738 Match Control Ulcer Col-F	1.1	0.0	117106 Normal Cartilage Rep20	4.9	9.2
112381 Ulcer Col-M	2.5	0.7	113663 Bone3 Normal	4.5	2.1
112735 Match Control Ulcer Col-M	9.5	7.6	113664 Synovium3 Normal	0.7	0.4
112382 Ulcer Col-M	50.0	48.6	113665 Syn Fluid Cells3 Normal	0.5	0.3
112394 Match Control Ulcer Col-M	1.1	0.0	117107 Normal Cartilage Rep22	2.0	4.5
112383 Ulcer Col-M	30.8	45.7	113667 Bone4 Normal	14.4	11.4
112736 Match Control Ulcer Col-M	69.3	40.3	113668 Synovium4 Normal	19.8	13.0
112423 Psoriasis-F	11.0	10.4	113669 Syn Fluid Cells4 Normal	36.9	33.7

Table 21C. Panel 1.3D

Tissue Name	Rel. Exp.(%) Ag2255, Run 148399930	Rel. Exp.(%) Ag2255, Run 148492532	Tissue Name	Rel. Exp.(%) Ag2255, Run 148399930	Rel. Exp.(%) Ag2255, Run 148492532
Liver adenocarcinoma	0.9	0.8	Kidney (fetal)	0.4	0.7
Pancreas	0.2	0.0	Renal ca. 786-0	0.0	0.0
Pancreatic ca. CAPAN 2	0.0	0.0	Renal ca. A498	0.0	0.0
Adrenal gland	0.4	0.4	Renal ca. RXF 393	0.0	0.0
Thyroid	0.7	0.4	Renal ca. ACHN	0.0	0.0
Salivary gland	0.2	0.6	Renal ca. UO-31	0.0	0.0
Pituitary gland	0.3	0.1	Renal ca. TK-10	0.0	0.0
Brain (fetal)	1.0	0.3	Liver	0.0	0.0
Brain (whole)	1.7	0.3	Liver (fetal)	0.0	0.0
Brain (amygdala)	0.2	0.3	Liver ca. (hepatoblast) HepG2	0.0	0.0
Brain (cerebellum)	1.4	0.8	Lung	0.0	0.9
Brain (hippocampus)	2.0	1.9	Lung (fetal)	2.6	1.7
Brain (substantia nigra)	1.3	0.0	Lung ca. (small cell) LX-1	26.4	24.0
Brain (thalamus)	1.6	1.2	Lung ca. (small cell) NCI-H69	2.2	2.0
Cerebral Cortex	1.4	1.6	Lung ca. (s.cell	0.2	0.4

			var.) SHP-77		
Spinal cord	0.8	0.4	Lung ca. (large cell)NCI-H460	1.1	1.7
glio/astro U87-MG	0.0	0.0	Lung ca. (non-sm. cell) A549	0.2	0.0
glio/astro U-118-MG	0.0	0.0	Lung ca. (non-s.cell) NCI-H23	1.4	1.4
astrocytoma SW1783	0.0	0.0	Lung ca. (non-s.cell) HOP-62	0.0	0.1
neuro*; met SK-N-AS	0.3	0.0	Lung ca. (non-s.cl) NCI-H522	0.0	0.0
astrocytoma SF-539	0.0	0.0	Lung ca. (squam.) SW 900	0.0	0.0
astrocytoma SNB-75	0.0	0.0	Lung ca. (squam.) NCI-H596	0.3	0.2
glioma SNB-19	0.0	0.0	Mammary gland	2.6	3.2
glioma U251	0.0	0.0	Breast ca.* (pl.ef) MCF-7	0.0	0.0
glioma SF-295	0.0	0.0	Breast ca.* (pl.ef) MDA-MB-231	0.0	0.0
Heart (fetal)	0.9	0.4	Breast ca.* (pl.ef) T47D	0.0	0.0
Heart	0.0	0.2	Breast ca. BT-549	0.0	0.0
Skeletal muscle (fetal)	**100.0**	**100.0**	Breast ca. MDA-N	0.0	0.0
Skeletal muscle	0.2	0.2	Ovary	2.5	2.3
Bone marrow	0.0	0.0	Ovarian ca. OVCAR-3	0.0	0.0
Thymus	0.5	0.1	Ovarian ca. OVCAR-4	0.0	0.0
Spleen	0.0	0.0	Ovarian ca. OVCAR-5	0.0	0.0
Lymph node	0.1	0.3	Ovarian ca. OVCAR-8	0.2	0.2
Colorectal	0.4	0.0	Ovarian ca. IGROV-1	0.0	0.0
Stomach	0.2	0.7	Ovarian ca.* (ascites) SK-OV-3	0.0	0.0
Small intestine	0.4	0.8	Uterus	1.4	1.3
Colon ca. SW480	0.4	0.9	Placenta	7.4	6.8
Colon ca.* SW620(SW480 met)	13.2	17.3	Prostate	3.1	2.8
Colon ca. HT29	0.0	0.0	Prostate ca.* (bone met)PC-3	0.4	0.0
Colon ca. HCT-116	0.0	0.4	Testis	0.8	0.3
Colon ca. CaCo-2	0.9	0.7	Melanoma Hs688(A).T	0.0	0.0
Colon ca. tissue(ODO3866)	0.1	0.6	Melanoma* (met) Hs688(B).T	0.3	0.4

Colon ca. HCC-2998	0.0	0.0	Melanoma UACC-62	0.0	0.0
Gastric ca.* (liver met) NCI-N87	0.0	0.0	Melanoma M14	0.0	0.0
Bladder	0.2	0.3	Melanoma LOX IMVI	0.3	0.0
Trachea	2.9	4.5	Melanoma* (met) SK-MEL-5	0.1	0.0
Kidney	0.0	0.0	Adipose	1.5	1.4

Table 21D. Panel 2D

Tissue Name	Rel. Exp.(%) Ag2255, Run 148399949	Rel. Exp.(%) Ag2255, Run 148492562	Tissue Name	Rel. Exp.(%) Ag2255, Run 148399949	Rel. Exp.(%) Ag2255, Run 148492562
Normal Colon	9.1	13.9	Kidney Margin 8120608	0.0	0.0
CC Well to Mod Diff (ODO3866)	3.3	2.0	Kidney Cancer 8120613	0.0	0.0
CC Margin (ODO3866)	2.4	2.0	Kidney Margin 8120614	1.9	0.4
CC Gr.2 rectosigmoid (ODO3868)	0.8	1.9	Kidney Cancer 9010320	1.4	0.8
CC Margin (ODO3868)	0.0	0.5	Kidney Margin 9010321	0.4	0.0
CC Mod Diff (ODO3920)	0.0	0.5	Normal Uterus	2.1	1.3
CC Margin (ODO3920)	0.4	0.8	Uterus Cancer 064011	**100.0**	85.3
CC Gr.2 ascend colon (ODO3921)	3.8	4.3	Normal Thyroid	5.2	8.4
CC Margin (ODO3921)	2.3	2.9	Thyroid Cancer 064010	1.0	0.4
CC from Partial Hepatectomy (ODO4309) Mets	0.4	0.0	Thyroid Cancer A302152	0.7	1.4
Liver Margin (ODO4309)	0.0	0.0	Thyroid Margin A302153	0.3	1.9
Colon mets to lung (OD04451-01)	0.0	0.4	Normal Breast	5.1	3.4
Lung Margin (OD04451-02)	3.3	2.8	Breast Cancer (OD04566)	1.1	2.4
Normal Prostate 6546-1	15.8	29.1	Breast Cancer (OD04590-01)	1.8	3.8
Prostate Cancer (OD04410)	3.4	7.2	Breast Cancer Mets (OD04590-03)	8.5	12.2
Prostate Margin (OD04410)	17.1	15.7	Breast Cancer Metastasis (OD04655-05)	0.9	1.0
Prostate Cancer (OD04720-01)	26.2	51.4	Breast Cancer 064006	7.5	6.1
Prostate Margin (OD04720-02)	61.1	69.7	Breast Cancer 1024	13.3	12.1
Normal Lung	13.5	15.4	Breast Cancer	3.2	4.5

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061010			9100266		
Lung Met to Muscle (ODO4286)	1.6	3.8	Breast Margin 9100265	7.0	6.2
Muscle Margin (ODO4286)	1.6	2.3	Breast Cancer A209073	10.7	15.6
Lung Malignant Cancer (OD03126)	28.9	29.5	Breast Margin A2090734	9.0	11.7
Lung Margin (OD03126)	8.7	7.9	Normal Liver	0.0	0.0
Lung Cancer (OD04404)	92.7	**100.0**	Liver Cancer 064003	0.3	0.0
Lung Margin (OD04404)	9.0	9.0	Liver Cancer 1025	0.0	0.0
Lung Cancer (OD04565)	9.7	7.0	Liver Cancer 1026	1.5	0.4
Lung Margin (OD04565)	1.6	0.8	Liver Cancer 6004-T	0.0	0.0
Lung Cancer (OD04237-01)	2.8	0.9	Liver Tissue 6004-N	0.2	0.0
Lung Margin (OD04237-02)	6.0	10.7	Liver Cancer 6005-T	0.5	0.0
Ocular Mel Met to Liver (ODO4310)	0.0	0.8	Liver Tissue 6005-N	0.0	0.0
Liver Margin (ODO4310)	0.0	0.0	Normal Bladder	1.4	0.8
Melanoma Mets to Lung (OD04321)	0.0	0.0	Bladder Cancer 1023	1.4	0.0
Lung Margin (OD04321)	4.0	7.2	Bladder Cancer A302173	7.5	12.1
Normal Kidney	2.7	1.8	Bladder Cancer (OD04718-01)	0.3	2.1
Kidney Ca, Nuclear grade 2 (OD04338)	0.6	1.3	Bladder Normal Adjacent (OD04718-03)	2.1	3.5
Kidney Margin (OD04338)	2.2	3.9	Normal Ovary	8.4	6.0
Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	0.0	Ovarian Cancer 064008	37.6	31.2
Kidney Margin (OD04339)	0.0	0.9	Ovarian Cancer (OD04768-07)	1.5	1.4
Kidney Ca, Clear cell type (OD04340)	0.6	0.0	Ovary Margin (OD04768-08)	5.3	5.3
Kidney Margin (OD04340)	1.0	0.5	Normal Stomach	1.8	3.3
Kidney Ca, Nuclear grade 3 (OD04348)	1.2	1.7	Gastric Cancer 9060358	3.8	2.0
Kidney Margin (OD04348)	0.8	0.4	Stomach Margin 9060359	1.9	1.4
Kidney Cancer (OD04622-01)	0.8	0.8	Gastric Cancer 9060395	11.3	12.2
Kidney Margin (OD04622-03)	0.4	0.8	Stomach Margin 9060394	2.9	3.5
Kidney Cancer	0.4	0.0	Gastric Cancer	2.4	5.4

(OD04450-01)			9060397		
Kidney Margin (OD04450-03)	0.5	0.7	Stomach Margin 9060396	1.9	3.0
Kidney Cancer 8120607	0.6	1.4	Gastric Cancer 064005	4.8	5.3

Table 21E. Panel 3D

Tissue Name	Rel. Exp.(%) Ag2255, Run 170745120	Tissue Name	Rel. Exp.(%) Ag2255, Run 170745120
Daoy- Medulloblastoma	0.0	Ca Ski- Cervical epidermoid carcinoma (metastasis)	1.6
TE671- Medulloblastoma	0.0	ES-2- Ovarian clear cell carcinoma	0.0
D283 Med- Medulloblastoma	0.0	Ramos- Stimulated with PMA/ionomycin 6h	0.0
PFSK-1- Primitive Neuroectodermal	0.0	Ramos- Stimulated with PMA/ionomycin 14h	0.0
XF-498- CNS	0.0	MEG-01- Chronic myelogenous leukemia (megokaryoblast)	0.0
SNB-78- Glioma	0.0	Raji- Burkitt's lymphoma	0.0
SF-268- Glioblastoma	0.0	Daudi- Burkitt's lymphoma	0.0
T98G- Glioblastoma	0.0	U266- B-cell plasmacytoma	0.0
SK-N-SH- Neuroblastoma (metastasis)	0.0	CA46- Burkitt's lymphoma	0.0
SF-295- Glioblastoma	0.0	RL- non-Hodgkin's B-cell lymphoma	0.0
Cerebellum	4.8	JM1- pre-B-cell lymphoma	0.0
Cerebellum	8.2	Jurkat- T cell leukemia	0.0
NCI-H292- Mucoepidermoid lung carcinoma	17.4	TF-1- Erythroleukemia	0.0
DMS-114- Small cell lung cancer	7.9	HUT 78- T-cell lymphoma	0.0
DMS-79- Small cell lung cancer	0.0	U937- Histiocytic lymphoma	0.0
NCI-H146- Small cell lung cancer	16.7	KU-812- Myelogenous leukemia	0.0
NCI-H526- Small cell lung cancer	12.3	769-P- Clear cell renal carcinoma	0.0
NCI-N417- Small cell lung cancer	0.0	Caki-2- Clear cell renal carcinoma	0.0
NCI-H82- Small cell lung cancer	0.9	SW 839- Clear cell renal carcinoma	0.0
NCI-H157- Squamous cell lung cancer (metastasis)	0.0	G401- Wilms' tumor	0.0
NCI-H1155- Large cell lung cancer	0.0	Hs766T- Pancreatic carcinoma (LN metastasis)	0.0
NCI-H1299- Large cell lung cancer	0.0	CAPAN-1- Pancreatic adenocarcinoma (liver metastasis)	0.0
NCI-H727- Lung carcinoid	94.6	SU86.86- Pancreatic carcinoma (liver metastasis)	0.0
NCI-UMC-11- Lung carcinoid	34.9	BxPC-3- Pancreatic adenocarcinoma	0.0
LX-1- Small cell lung cancer	**100.0**	HPAC- Pancreatic adenocarcinoma	0.0
Colo-205- Colon cancer	18.4	MIA PaCa-2- Pancreatic carcinoma	0.0

KM12- Colon cancer	0.0	CFPAC-1- Pancreatic ductal adenocarcinoma	9.0
KM20L2- Colon cancer	0.0	PANC-1- Pancreatic epithelioid ductal carcinoma	0.0
NCI-H716- Colon cancer	0.0	T24- Bladder carcinma (transitional cell)	0.0
SW-48- Colon adenocarcinoma	71.7	5637- Bladder carcinoma	0.5
SW1116- Colon adenocarcinoma	8.5	HT-1197- Bladder carcinoma	0.0
LS 174T- Colon adenocarcinoma	0.0	UM-UC-3- Bladder carcinma (transitional cell)	0.0
SW-948- Colon adenocarcinoma	0.0	A204- Rhabdomyosarcoma	0.0
SW-480- Colon adenocarcinoma	0.0	HT-1080- Fibrosarcoma	0.0
NCI-SNU-5- Gastric carcinoma	0.0	MG-63- Osteosarcoma	0.0
KATO III- Gastric carcinoma	0.0	SK-LMS-1- Leiomyosarcoma (vulva)	0.0
NCI-SNU-16- Gastric carcinoma	0.0	SJRH30- Rhabdomyosarcoma (met to bone marrow)	0.0
NCI-SNU-1- Gastric carcinoma	0.0	A431- Epidermoid carcinoma	21.6
RF-1- Gastric adenocarcinoma	0.0	WM266-4- Melanoma	0.0
RF-48- Gastric adenocarcinoma	0.0	DU 145- Prostate carcinoma (brain metastasis)	0.0
MKN-45- Gastric carcinoma	0.0	MDA-MB-468- Breast adenocarcinoma	0.0
NCI-N87- Gastric carcinoma	0.0	SCC-4- Squamous cell carcinoma of tongue	0.0
OVCAR-5- Ovarian carcinoma	0.0	SCC-9- Squamous cell carcinoma of tongue	0.0
RL95-2- Uterine carcinoma	62.0	SCC-15- Squamous cell carcinoma of tongue	0.0
HelaS3- Cervical adenocarcinoma	41.2	CAL 27- Squamous cell carcinoma of tongue	0.0

Table 21F. Panel 4D

Tissue Name	Rel. Exp.(%) Ag2255, Run 148492583	Rel. Exp.(%) Ag2255, Run 152572164	Tissue Name	Rel. Exp.(%) Ag2255, Run 148492583	Rel. Exp.(%) Ag2255, Run 152572164
Secondary Th1 act	0.0	0.0	HUVEC IL-1beta	0.0	0.0
Secondary Th2 act	0.0	0.0	HUVEC IFN gamma	0.0	0.0
Secondary Tr1 act	0.4	0.0	HUVEC TNF alpha + IFN gamma	0.0	0.0
Secondary Th1 rest	0.0	0.0	HUVEC TNF alpha + IL4	0.0	0.0
Secondary Th2 rest	0.3	0.8	HUVEC IL-11	0.0	0.0
Secondary Tr1 rest	0.0	0.0	Lung Microvascular EC none	0.0	0.0
Primary Th1 act	0.0	0.0	Lung Microvascular EC TNFalpha + IL-	0.0	0.0

			1beta		
Primary Th2 act	0.0	0.0	Microvascular Dermal EC none	0.0	0.3
Primary Tr1 act	0.0	0.0	Microsvasular Dermal EC TNFalpha + IL-1beta	0.0	0.0
Primary Th1 rest	0.0	0.0	Bronchial epithelium TNFalpha + IL1beta	1.4	0.3
Primary Th2 rest	0.0	0.0	Small airway epithelium none	0.6	1.2
Primary Tr1 rest	0.0	0.0	Small airway epithelium TNFalpha + IL-1beta	3.6	3.0
CD45RA CD4 lymphocyte act	0.0	0.0	Coronery artery SMC rest	0.0	0.0
CD45RO CD4 lymphocyte act	0.0	0.0	Coronery artery SMC TNFalpha + IL-1beta	0.8	0.0
CD8 lymphocyte act	0.0	0.0	Astrocytes rest	0.0	0.0
Secondary CD8 lymphocyte rest	0.0	0.0	Astrocytes TNFalpha + IL-1beta	0.0	0.0
Secondary CD8 lymphocyte act	0.0	0.0	KU-812 (Basophil) rest	0.0	0.0
CD4 lymphocyte none	0.0	0.0	KU-812 (Basophil) PMA/ionomycin	0.0	0.0
2ry Th1/Th2/Tr1_anti-CD95 CH11	0.0	0.0	CCD1106 (Keratinocytes) none	17.2	14.0
LAK cells rest	0.6	0.0	CCD1106 (Keratinocytes) TNFalpha + IL-1beta	2.4	0.8
LAK cells IL-2	0.0	0.0	Liver cirrhosis	2.4	2.4
LAK cells IL-2+IL-12	0.0	0.0	Lupus kidney	0.0	0.0
LAK cells IL-2+IFN gamma	0.0	0.0	NCI-H292 none	12.2	14.2
LAK cells IL-2+ IL-18	0.0	0.0	NCI-H292 IL-4	**100.0**	**100.0**
LAK cells PMA/ionomycin	0.0	0.0	NCI-H292 IL-9	11.3	14.3
NK Cells IL-2 rest	0.0	0.0	NCI-H292 IL-13	47.3	65.5
Two Way MLR 3 day	0.0	0.0	NCI-H292 IFN gamma	8.1	8.1
Two Way MLR 5 day	0.0	0.0	HPAEC none	0.0	0.0
Two Way MLR 7 day	0.0	0.0	HPAEC TNF alpha + IL-1 beta	0.0	0.0
PBMC rest	0.0	0.0	Lung fibroblast none	0.0	0.0
PBMC PWM	0.0	0.0	Lung fibroblast TNF alpha + IL-1 beta	0.0	0.0
PBMC PHA-L	0.0	0.4	Lung fibroblast IL-4	0.0	0.0
Ramos (B cell) none	0.0	0.0	Lung fibroblast IL-9	0.0	0.0
Ramos (B cell) ionomycin	0.0	0.0	Lung fibroblast IL-13	0.2	0.0
B lymphocytes PWM	0.0	0.4	Lung fibroblast IFN	0.0	0.0

			gamma		
B lymphocytes CD40L and IL-4	0.2	0.3	Dermal fibroblast CCD1070 rest	0.0	0.3
EOL-1 dbcAMP	0.0	0.0	Dermal fibroblast CCD1070 TNF alpha	0.0	0.0
EOL-1 dbcAMP PMA/ionomycin	0.4	0.0	Dermal fibroblast CCD1070 IL-1 beta	0.0	0.0
Dendritic cells none	0.0	0.0	Dermal fibroblast IFN gamma	0.3	0.0
Dendritic cells LPS	0.0	0.0	Dermal fibroblast IL-4	2.1	0.3
Dendritic cells anti-CD40	0.0	0.0	IBD Colitis 2	0.7	0.4
Monocytes rest	0.0	0.0	IBD Crohn's	0.3	0.0
Monocytes LPS	0.0	0.4	Colon	6.1	6.3
Macrophages rest	0.0	0.0	Lung	9.7	15.2
Macrophages LPS	0.0	0.0	Thymus	1.6	0.9
HUVEC none	0.0	0.0	Kidney	1.5	1.7
HUVEC starved	0.0	0.0			

AI_comprehensive panel_v1.0 Summary: Ag2255 Two experiments with the same probe and primer set both show highest expression of the CG50379-01 gene, a Frizzled-10 homolog, in samples from a subset of emphysema patients (CTs=31-32). In addition, this gene

5   is also expressed at moderate levels in samples from the mucoepidermoid pulmonary epithelial cell line NCI-H292 activated with IL-4 or IL-13 in culture. Based on this expression profile, small molecule drugs or antibodies that antagonize the action of the putative 7-transmembrane receptor encoded by the CG50379-01 gene may be useful as therapeutics which reduce or eliminate the symptoms in patients with allergy, asthma, or chronic obstructive pulmonary

10   diseases.

Panel 1.3D Summary: Ag2255 Two experiments with the same probe and primer set both show highest expression of the CG50379-01 gene, a Frizzled-10 homolog, in samples derived from fetal skeletal muscle (CTs=29-30). Furthermore, expression in fetal skeletal muscle is significantly higher than in adult skeletal muscle (CTs=38). Thus, expression of this

15   gene could be used to differentiate between fetal and adult skeletal muscle. In addition, the significantly higher levels of expression in fetal skeletal muscle suggest that this gene product may enhance muscular growth or development in the fetus and thus may also act in a regenerative capacity in the adult. Therefore, therapeutic modulation of the protein encoded by this gene could be useful in treatment of muscle related diseases. More specifically, treatment

20   of weak or dystrophic muscle with the protein encoded by this gene could restore muscle mass or function.

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The CG50379-01 gene is also expressed in small cell lung cancer and colon carcinoma. Therefore, expression of this gene could be used to differentiate between these samples and other samples on this panel. Furthermeore, therapeutic targeting of FZD10 with a monoclonal antibody is anticipated to limit or block the extent of tumor cell migration, invasion and
5   growth, specifically in lung and colon tumors.

References:

Koike J, Takagi A, Miwa T, Hirai M, Terada M, Katoh M Molecular cloning of Frizzled-10, a novel member of the Frizzled gene family. Biochem Biophys Res Commun 1999 Aug 19;262(1):39-43

10   In the above reference, the Frizzled genes are found to encode WNT receptors. Frizzled-10 (FZD10) was cloned and characterized. Nucleotide sequence analysis showed that human FZD10 gene encodes a seven-transmembrane-receptor of 581 amino acids, with an N-terminal cysteine-rich domain and a C-terminal Ser/Thr-Xxx-Val motif. Larger amounts of FZD10 mRNA, 4.0 kb in size, were detected in the placenta and fetal kidney, followed by fetal
15   lung and brain. In adult brain, FZD10 mRNA was abundant in the cerebellum. Among cancer cell lines, FZD10 was highly expressed in a cervical cancer cell line, HeLa S3, and moderately in a colon cancer cell line, SW480. The FZD10 gene was mapped to human chromosome 12q24.33.

Kawakami Y, Wada N, Nishimatsu S, Nohno T Involvement of frizzled-10 in Wnt-7a
20   signaling during chick limb development. Dev Growth Differ 2000 Dec;42(6):561-9

Abstract: The dorsal ectoderm of the limb bud is known to regulate anterior-posterior patterning as well as dorsal-ventral patterning during vertebrate limb morphogenesis. Wnt-7a, expressed in the dorsal ectoderm, encodes a key molecule implicated in these events. In the present study, chicken frizzled-10 (Fz-10) encoding a Wnt receptor was used to study
25   mechanisms of Wnt-7a signaling during chick limb patterning, because its expression is restricted to the posterior-distal region of the dorsal limb bud. Fz-10 transcripts colocalize with Sonic hedgehog (Shh) in the dorsal side of stages 18-23 chick limb buds. It was demonstrated that Fz-10 interacts with Wnt-7a to induce synergistically the expression of Wnt-responsive genes, such as Siamois and Xnr3, in Xenopus animal cap assays. In the chick limb bud, Fz-10
30   expression is regulated by Shh and a signal from the dorsal ectoderm, presumably Wnt-7a, but not by signals from the apical ectodermal ridge. These results suggest that Fz-10 acts as a receptor for Wnt-7a and has a positive effect on Shh expression in the chick limb bud.

Panel 2D Summary: Ag2255 Two experiments with the same probe and primer set both show highest expression of the CG50379-01 gene, a Frizzled-10 homolog, in samples derived from uterine and lung cancer (CTs=30-31). Significant expression is also seen in prostate cancer. In addition, this gene is overexpressed in uterine, lung and gastric tumors compared with their normal adjacent tissue. Therefore, therapeutic targeting of FZD10 with a monoclonal antibody is anticipated to limit or block the extent of tumor cell migration, invasion and growth, preferably in lung, uterine, prostate, gastric and ovarian tumors.

Panel 3D Summary: Ag2255 The expression of the CG50379-01 gene appears to be highest in a sample derived from a lung cancer cell line (LX-1)(CT=30.7). There also appears to be substantial expression in other lung cancer cell lines as well as colon cancer cell lines. This expression is consistent with the expression seen in Panel 1.3D. Thus, the expression of this gene could be used to distinguish LX-1 samples from other samples in the panel. Moreover, therapeutic modulation of thie gene, through the use of small molecule drugs, antibodies or protein therapeutics might be beneficial in the treatment of lung or colon cancer.

Panel 4D Summary: Ag2255 Two experiments with the same probe and primer set both show highest expression of the CG50379-01 gene, a Frizzled-10 homolog, in samples derived from the pulmonary mucoepidermoid cell line NCI-H292 stimulated with IL-4 (CTs=30). Significant expression is also seen in IL-13 activated NCI-H292. This prominent expression in lung-derived tissue is consistent with the previous panels and particularly expression in this cell line is consistent with the expression in AI_comprehensive panel_v1.0. Thus, this expression profile indicates that this gene product may play a key role as a mediator of inflammation, especially in late-phase allergic reactions, and as a mediator of local cellular movement or trafficking into the inflamed area by cytokines and chemokines. Therefore, therapeutic targeting of CG50379-01 with a monoclonal antibody or small molecule drug that antagonize the action of this 7-membrane receptor homolog is anticipated to limit or block the extent of inflammation potential and thus the symptoms, caused by pro-inflammatory cytokines such as IL-4 or IL-13, when these cytokines are induced in allergic, asthma and COPD patients.

References:

Louahed J, Toda M, Jen J, Hamid Q, Renauld JC, Levitt RC, Nicolaides NC Interleukin-9 upregulates mucus expression in the airways. Am J Respir Cell Mol Biol 2000 Jun;22(6):649-56

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Abstract: Interleukin (IL)-9 has recently been shown to play an important role in allergic disease because its expression is strongly associated with the degree of airway responsiveness and the asthmatic-like phenotype. IL-9 is a pleiotropic cytokine that is active on many cell types involved in the allergic immune response. Mucus hypersecretion is a

5   clinical feature of chronic airway diseases; however, the mechanisms underlying the induction of mucin are poorly understood. In this report, it is shown that IL-9 regulates the expression of a subset of mucin genes in lung cells both in vivo and in vitro. In vivo, the constitutive expression of IL-9 in transgenic mice results in elevated MUC2 and MUC5AC gene expression in airway epithelial cells and periodic acid-Schiff-positive staining (reflecting

10   mucous glycogenates). Similar results were observed in C57BL/6J mice after IL-9 intratracheal instillation. In contrast, instillation of the T helper 1-associated cytokine interferon gamma failed to induce mucin production. In vitro, our studies showed that IL-9 also induces expression of MUC2 and MUC5AC in human primary lung cultures and in the human muccoepidermoid NCI-H292 cell line, indicating a direct effect of IL-9 on inducing

15   mucin expression in these cells. Altogether, these results suggest that upregulation of mucin by IL-9 might contribute to the pathogenesis of human inflammatory airway disorders, such as asthma. These data extend the role of the biologic processes that IL-9 has on regulating the many clinical features of asthma and further supports the IL-9 pathway as a key mediator of the asthmatic response.

20   Zheng T, Zhu Z, Wang Z, Homer RJ, Ma B, Riese RJ Jr, Chapman HA Jr, Shapiro SD, Elias JA Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. J Clin Invest 2000 Nov;106(9):1081-93

Abstract: Cigarette smoke exposure is the major cause of chronic obstructive pulmonary disease (COPD). However, only a minority of smokers develop significant COPD,

25   and patients with asthma or asthma-like airway hyperresponsiveness or eosinophilia experience accelerated loss of lung function after cigarette smoke exposure. Pulmonary inflammation is a characteristic feature of lungs from patients with COPD. Surprisingly, the mediators of this inflammation and their contributions to the pathogenesis and varied natural history of COPD are not well defined. Here it is shown that IL-13, a critical cytokine in

30   asthma, causes emphysema with enhanced lung volumes and compliance, mucus metaplasia, and inflammation, when inducibly overexpressed in the adult murine lung. MMP-2, -9, -12, - 13, and -14 and cathepsins B, S, L, H, and K were induced by IL-13 in this setting. In addition, treatment with MMP or cysteine proteinase antagonists significantly decreased the emphysema and inflammation, but not the mucus in these animals. These studies demonstrate

that IL-13 is a potent stimulator of MMP and cathepsin-based proteolytic pathways in the lung. They also demonstrate that IL-13 causes emphysema via a MMP- and cathepsin-dependent mechanism(s) and highlight common mechanisms that may underlie COPD and asthma.

5   **SEC4 (CG55023-01/SC46872089)**

Expression of gene CG55023-01 was assessed using the primer-probe sets Ag692, Ag264 and Ag264b, described in Tables 22A, 22B and 22C. Results of the RTQ-PCR runs are shown in Tables 22D, 22E, 22F, 22G and 22H.

Table 22A. Probe Name Ag692

Primers	Sequences	Length	Start Position
Forward	5'-cttgaagttctcacacctttgc-3' (SEQ ID NO:144)	22	207
Probe	TET-5'-tcataacagttactgcatcaacggtg-3'-TAMRA (SEQ ID NO:145)	26	237
Reverse	5'-tcatggtggaatgcacaag-3' (SEQ ID NO:146)	19	263

10   Table 22B. Probe Name Ag264

Primers	Sequences	Length	Start Position
Forward	5'-gtctatcttttattcaacgcaatgaca-3' (SEQ ID NO:147)	27	73
Probe	TET-5'-agtcacggctgcctcttcggtca-3'-TAMRA (SEQ ID NO:148)	23	104
Reverse	5'-gggctgtgattggaggtgtt-3' (SEQ ID NO:149)	20	129

Table 22C. Probe Name Ag264b

Primers	Sequences	Length	Start Position
Forward	5'-gtctatcttttattcaacgcaatgaca-3' (SEQ ID NO:150)	27	73
Probe	TET-5'-cacggctgcctcttcggtcagtg-3'-TAMRA (SEQ ID NO:151)	23	101
Reverse	5'-gggctgtgattggaggtgtta-3' (SEQ ID NO:152)	21	128

Table 22D. CNS_neurodegeneration_v1.0

Tissue Name	Rel. Exp.(%) Ag692, Run 224996549	Tissue Name	Rel. Exp.(%) Ag692, Run 224996549
AD 1 Hippo	0.0	Control (Path) 3 Temporal Ctx	15.4
AD 2 Hippo	5.6	Control (Path) 4 Temporal Ctx	17.6
AD 3 Hippo	0.0	AD 1 Occipital Ctx	21.0
AD 4 Hippo	0.0	AD 2 Occipital Ctx (Missing)	0.0
AD 5 hippo	69.7	AD 3 Occipital Ctx	31.9
AD 6 Hippo	70.2	AD 4 Occipital Ctx	22.4
Control 2 Hippo	61.6	AD 5 Occipital Ctx	24.3
Control 4 Hippo	27.2	AD 6 Occipital Ctx	75.8
Control (Path) 3 Hippo	0.0	Control 1 Occipital Ctx	20.7
AD 1 Temporal Ctx	0.0	Control 2 Occipital Ctx	41.8
AD 2 Temporal Ctx	37.4	Control 3 Occipital Ctx	21.9
AD 3 Temporal Ctx	0.0	Control 4 Occipital Ctx	0.0
AD 4 Temporal Ctx	10.5	Control (Path) 1	75.8

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		Occipital Ctx	
AD 5 Inf Temporal Ctx	81.2	Control (Path) 2 Occipital Ctx	0.0
AD 5 SupTemporal Ctx	92.7	Control (Path) 3 Occipital Ctx	0.0
AD 6 Inf Temporal Ctx	23.5	Control (Path) 4 Occipital Ctx	0.0
AD 6 Sup Temporal Ctx	69.3	Control 1 Parietal Ctx	30.8
Control 1 Temporal Ctx	66.0	Control 2 Parietal Ctx	42.6
Control 2 Temporal Ctx	44.8	Control 3 Parietal Ctx	18.3
Control 3 Temporal Ctx	0.0	Control (Path) 1 Parietal Ctx	53.6
Control 4 Temporal Ctx	21.2	Control (Path) 2 Parietal Ctx	22.2
Control (Path) 1 Temporal Ctx	64.2	Control (Path) 3 Parietal Ctx	0.0
Control (Path) 2 Temporal Ctx	**100.0**	Control (Path) 4 Parietal Ctx	25.5

Table 22E. Panel 1

Tissue Name	Rel. Exp.(%) Ag264, Run 87590466	Rel. Exp.(%) Ag264, Run 88794920	Rel. Exp.(%) Ag264b, Run 97806010	Tissue Name	Rel. Exp.(%) Ag264, Run 87590466	Rel. Exp.(%) Ag264, Run 88794920	Rel. Exp.(%) Ag264b, Run 97806010
Endothelial cells	0.0	0.0	2.5	Renal ca. 786-0	0.0	0.0	2.4
Endothelial cells (treated)	0.0	0.0	2.6	Renal ca. A498	0.0	0.0	2.3
Pancreas	0.0	0.0	3.5	Renal ca. RXF 393	0.0	0.0	3.1
Pancreatic ca. CAPAN 2	0.0	0.0	2.2	Renal ca. ACHN	0.0	0.0	3.5
Adrenal gland	0.0	0.0	3.6	Renal ca. UO-31	0.0	0.0	2.5
Thyroid	0.0	0.0	3.7	Renal ca. TK-10	0.0	0.0	2.3
Salivary gland	0.0	0.0	2.8	Liver	0.0	0.0	2.6
Pituitary gland	0.0	0.0	2.4	Liver (fetal)	0.0	0.0	3.0
Brain (fetal)	0.0	0.0	3.2	Liver ca. (hepatoblast) HepG2	0.0	0.0	2.2
Brain (whole)	0.0	0.0	2.5	Lung	0.0	0.0	4.5
Brain (amygdala)	0.0	0.0	2.7	Lung (fetal)	0.0	0.0	3.4
Brain (cerebellum)	0.0	0.0	3.2	Lung ca. (small cell) LX-1	0.0	0.0	3.0
Brain (hippocampus)	0.0	0.0	4.6	Lung ca. (small cell) NCI-H69	0.0	0.0	2.4
Brain (substantia nigra)	0.0	0.0	3.1	Lung ca. (s.cell var.) SHP-77	0.0	0.0	2.0

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Brain (thalamus)	0.0	0.0	4.0	Lung ca. (large cell)NCI-H460	37.1	39.8	68.8
Brain (hypothalamus)	0.0	0.0	4.7	Lung ca. (non-sm. cell) A549	0.0	0.0	2.1
Spinal cord	0.0	0.0	2.9	Lung ca. (non-s.cell) NCI-H23	0.0	0.0	3.7
glio/astro U87-MG	0.0	0.0	3.3	Lung ca. (non-s.cell) HOP-62	0.0	0.0	3.2
glio/astro U-118-MG	0.0	0.0	2.4	Lung ca. (non-s.cl) NCI-H522	0.0	0.0	2.9
astrocytoma SW1783	0.0	0.0	3.1	Lung ca. (squam.) SW 900	0.0	0.0	3.4
neuro*; met SK-N-AS	0.0	0.0	3.0	Lung ca. (squam.) NCI-H596	0.0	0.0	2.9
astrocytoma SF-539	0.0	0.0	2.3	Mammary gland	0.0	0.0	2.9
astrocytoma SNB-75	0.0	0.0	2.6	Breast ca.* (pl.ef) MCF-7	0.0	0.0	2.1
glioma SNB-19	0.0	0.0	2.8	Breast ca.* (pl.ef) MDA-MB-231	0.0	0.0	2.9
glioma U251	0.0	0.0	2.1	Breast ca.* (pl. ef) T47D	0.0	0.0	3.8
glioma SF-295	0.0	0.0	4.1	Breast ca. BT-549	0.0	0.0	2.0
Heart	0.0	0.0	4.5	Breast ca. MDA-N	0.0	0.0	2.4
Skeletal muscle	0.0	0.0	2.8	Ovary	0.0	0.0	3.0
Bone marrow	0.0	0.0	2.8	Ovarian ca. OVCAR-3	0.0	0.0	6.9
Thymus	0.0	0.0	4.6	Ovarian ca. OVCAR-4	0.0	0.0	2.2
Spleen	0.0	0.0	2.2	Ovarian ca. OVCAR-5	0.0	0.0	2.7
Lymph node	0.0	0.0	2.4	Ovarian ca. OVCAR-8	0.0	0.0	3.0
Colon (ascending)	0.0	0.0	2.0	Ovarian ca. IGROV-1	0.0	0.0	2.6
Stomach	0.0	0.0	3.6	Ovarian ca. (ascites) SK-OV-3	0.0	0.0	2.8
Small intestine	0.0	0.0	2.5	Uterus	0.0	0.0	2.3
Colon ca. SW480	0.0	0.0	2.4	Placenta	0.0	0.0	4.7
Colon ca.* SW620 (SW480 met)	0.0	0.0	2.7	Prostate	0.0	0.0	2.4
Colon ca. HT29	0.0	0.0	2.5	Prostate ca.* (bone met)	**100.0**	**100.0**	**100.0**

				PC-3			
Colon ca. HCT-116	0.0	0.0	2.8	Testis	0.0	10.0	5.3
Colon ca. CaCo-2	0.0	0.0	4.0	Melanoma Hs688(A).T	0.0	0.0	2.4
Colon ca. HCT-15	0.0	0.0	2.0	Melanoma* (met) Hs688(B).T	0.0	0.0	3.7
Colon ca. HCC-2998	0.0	0.0	2.6	Melanoma UACC-62	0.0	0.0	3.1
Gastric ca. * (liver met) NCI-N87	0.0	0.0	3.1	Melanoma M14	0.0	0.0	2.4
Bladder	0.0	0.0	2.8	Melanoma LOX IMVI	0.0	0.0	3.3
Trachea	0.0	0.0	2.7	Melanoma* (met) SK-MEL-5	0.0	0.0	2.5
Kidney	0.0	0.0	2.4	Melanoma SK-MEL-28	0.0	0.0	2.6
Kidney (fetal)	0.0	0.0	3.3				

Table 22F. Panel 1.2

Tissue Name	Rel. Exp.(%) Ag692, Run 114250175	Rel. Exp.(%) Ag692, Run 117052376	Tissue Name	Rel. Exp.(%) Ag692, Run 114250175	Rel. Exp.(%) Ag692, Run 117052376
Endothelial cells	2.9	0.0	Renal ca. 786-0	1.6	0.4
Heart (Fetal)	0.2	0.0	Renal ca. A498	1.1	0.0
Pancreas	4.5	0.1	Renal ca. RXF 393	0.1	0.0
Pancreatic ca. CAPAN 2	2.3	0.4	Renal ca. ACHN	3.4	0.6
Adrenal Gland	1.6	0.1	Renal ca. UO-31	4.5	0.1
Thyroid	0.9	0.0	Renal ca. TK-10	8.0	2.2
Salivary gland	3.8	0.7	Liver	1.0	0.0
Pituitary gland	1.3	0.0	Liver (fetal)	1.1	0.3
Brain (fetal)	1.0	0.0	Liver ca. (hepatoblast) HepG2	1.3	0.0
Brain (whole)	1.0	0.0	Lung	6.7	5.1
Brain (amygdala)	0.2	0.0	Lung (fetal)	1.5	0.9
Brain (cerebellum)	1.4	0.0	Lung ca. (small cell) LX-1	6.5	1.2
Brain (hippocampus)	0.4	0.0	Lung ca. (small cell) NCI-H69	0.9	0.1
Brain (thalamus)	0.6	0.0	Lung ca. (s.cell var.) SHP-77	0.4	0.0
Cerebral Cortex	2.0	0.0	Lung ca. (large cell)NCI-H460	41.8	36.3
Spinal cord	2.6	0.3	Lung ca. (non-sm. cell) A549	3.3	0.8
glio/astro U87-MG	13.5	6.5	Lung ca. (non-s.cell) NCI-H23	1.1	0.0
glio/astro U-118-	2.2	0.9	Lung ca. (non-	6.8	3.7

MG			s.cell) HOP-62		
astrocytoma SW1783	1.6	0.9	Lung ca. (non-s.cl) NCI-H522	20.6	10.1
neuro*; met SK-N-AS	4.9	0.0	Lung ca. (squam.) SW 900	10.4	6.5
astrocytoma SF-539	0.5	0.0	Lung ca. (squam.) NCI-H596	1.5	0.0
astrocytoma SNB-75	0.1	0.0	Mammary gland	5.5	0.8
glioma SNB-19	4.4	1.0	Breast ca.* (pl.ef) MCF-7	0.4	0.0
glioma U251	0.8	0.0	Breast ca.* (pl.ef) MDA-MB-231	2.2	0.8
glioma SF-295	4.0	0.0	Breast ca.* (pl. ef) T47D	4.0	1.9
Heart	5.3	3.4	Breast ca. BT-549	0.7	0.1
Skeletal Muscle	1.4	0.0	Breast ca. MDA-N	15.0	0.0
Bone marrow	1.7	2.1	Ovary	0.2	0.0
Thymus	0.0	0.2	Ovarian ca. OVCAR-3	16.3	6.9
Spleen	0.6	0.0	Ovarian ca. OVCAR-4	0.5	0.0
Lymph node	0.8	0.0	Ovarian ca. OVCAR-5	6.9	2.6
Colorectal Tissue	0.1	0.0	Ovarian ca. OVCAR-8	0.2	0.0
Stomach	3.7	1.8	Ovarian ca. IGROV-1	12.6	8.5
Small intestine	1.1	0.1	Ovarian ca. (ascites) SK-OV-3	3.1	0.9
Colon ca. SW480	0.3	0.0	Uterus	0.4	0.0
Colon ca.* SW620 (SW480 met)	1.9	0.0	Placenta	6.5	4.7
Colon ca. HT29	2.2	0.3	Prostate	0.8	0.0
Colon ca. HCT-116	3.4	1.4	Prostate ca.* (bone met) PC-3	**100.0**	**100.0**
Colon ca. CaCo-2	1.2	0.2	Testis	8.7	8.0
Colon ca. Tissue (ODO3866)	0.3	0.0	Melanoma Hs688(A).T	0.5	0.0
Colon ca. HCC-2998	5.8	2.0	Melanoma* (met) Hs688(B).T	1.4	0.7
Gastric ca.* (liver met) NCI-N87	11.9	2.9	Melanoma UACC-62	1.0	0.0
Bladder	2.5	0.8	Melanoma M14	4.0	0.0
Trachea	1.2	0.0	Melanoma LOX IMVI	1.5	0.3
Kidney	2.3	0.0	Melanoma* (met) SK-MEL-5	5.1	0.0
Kidney (fetal)	2.5	0.2			

Table 22G. Panel 2D

Tissue Name	Rel. Exp.(%) Ag264, Run 144872209	Rel. Exp.(%) Ag692, Run 145177146	Tissue Name	Rel. Exp.(%) Ag264, Run 144872209	Rel. Exp.(%) Ag692, Run 145177146
Normal Colon	3.1	3.2	Kidney Margin 8120608	0.0	0.0
CC Well to Mod Diff (ODO3866)	0.0	0.3	Kidney Cancer 8120613	0.0	0.0
CC Margin (ODO3866)	0.0	1.0	Kidney Margin 8120614	0.0	0.0
CC Gr.2 rectosigmoid (ODO3868)	0.7	0.0	Kidney Cancer 9010320	0.0	0.0
CC Margin (ODO3868)	0.0	0.6	Kidney Margin 9010321	0.0	0.3
CC Mod Diff (ODO3920)	0.0	0.0	Normal Uterus	0.0	0.0
CC Margin (ODO3920)	0.0	0.3	Uterus Cancer 064011	0.0	0.5
CC Gr.2 ascend colon (ODO3921)	0.0	0.7	Normal Thyroid	0.2	0.0
CC Margin (ODO3921)	0.0	1.3	Thyroid Cancer 064010	0.0	0.3
CC from Partial Hepatectomy (ODO4309) Mets	0.0	0.3	Thyroid Cancer A302152	0.3	0.0
Liver Margin (ODO4309)	0.0	0.0	Thyroid Margin A302153	0.0	0.3
Colon mets to lung (OD04451-01)	0.3	1.0	Normal Breast	2.6	2.4
Lung Margin (OD04451-02)	4.5	1.3	Breast Cancer (OD04566)	0.0	0.0
Normal Prostate 6546-1	0.0	1.4	Breast Cancer (OD04590-01)	0.0	0.0
Prostate Cancer (OD04410)	0.5	0.9	Breast Cancer Mets (OD04590-03)	0.3	0.0
Prostate Margin (OD04410)	0.0	0.3	Breast Cancer Metastasis (OD04655-05)	0.0	0.3
Prostate Cancer (OD04720-01)	0.4	0.3	Breast Cancer 064006	11.9	7.4
Prostate Margin (OD04720-02)	1.7	2.7	Breast Cancer 1024	3.1	3.1
Normal Lung 061010	0.3	1.1	Breast Cancer 9100266	0.4	0.0
Lung Met to Muscle (ODO4286)	100.0	98.6	Breast Margin 9100265	0.0	0.3
Muscle Margin (ODO4286)	0.0	0.9	Breast Cancer A209073	22.7	16.2
Lung Malignant Cancer (OD03126)	0.3	0.9	Breast Margin A2090734	2.2	1.6
Lung Margin (OD03126)	0.8	2.3	Normal Liver	0.0	0.7
Lung Cancer	81.2	100.0	Liver Cancer	0.0	0.5

(OD04404)			064003		
Lung Margin (OD04404)	3.7	3.8	Liver Cancer 1025	0.0	0.3
Lung Cancer (OD04565)	5.8	3.8	Liver Cancer 1026	0.0	0.0
Lung Margin (OD04565)	0.0	0.7	Liver Cancer 6004-T	0.0	0.7
Lung Cancer (OD04237-01)	0.7	1.0	Liver Tissue 6004-N	0.0	0.1
Lung Margin (OD04237-02)	4.9	8.8	Liver Cancer 6005-T	0.0	0.2
Ocular Mel Met to Liver (ODO4310)	0.0	0.0	Liver Tissue 6005-N	0.0	0.0
Liver Margin (ODO4310)	0.0	0.5	Normal Bladder	0.7	0.0
Melanoma Mets to Lung (OD04321)	0.0	0.0	Bladder Cancer 1023	0.0	0.0
Lung Margin (OD04321)	8.8	7.5	Bladder Cancer A302173	50.7	48.6
Normal Kidney	0.0	1.0	Bladder Cancer (OD04718-01)	7.3	7.0
Kidney Ca, Nuclear grade 2 (OD04338)	1.6	1.6	Bladder Normal Adjacent (OD04718-03)	0.3	0.5
Kidney Margin (OD04338)	0.0	0.6	Normal Ovary	0.0	0.0
Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	1.8	Ovarian Cancer 064008	3.8	5.6
Kidney Margin (OD04339)	0.2	0.6	Ovarian Cancer (OD04768-07)	0.0	0.0
Kidney Ca, Clear cell type (OD04340)	0.3	1.3	Ovary Margin (OD04768-08)	34.2	28.1
Kidney Margin (OD04340)	0.8	2.4	Normal Stomach	0.0	0.0
Kidney Ca, Nuclear grade 3 (OD04348)	0.0	0.0	Gastric Cancer 9060358	0.0	0.0
Kidney Margin (OD04348)	1.4	0.8	Stomach Margin 9060359	0.0	0.0
Kidney Cancer (OD04622-01)	0.3	0.3	Gastric Cancer 9060395	0.3	0.5
Kidney Margin (OD04622-03)	0.3	0.3	Stomach Margin 9060394	0.0	0.0
Kidney Cancer (OD04450-01)	0.0	0.0	Gastric Cancer 9060397	0.0	0.0
Kidney Margin (OD04450-03)	0.0	0.6	Stomach Margin 9060396	0.0	0.0
Kidney Cancer 8120607	0.3	0.0	Gastric Cancer 064005	0.0	0.2

Table 22H. Panel 4D

Tissue Name	Rel. Exp.(%) Ag692, Run 164318656	Tissue Name	Rel. Exp.(%) Ag692, Run 164318656
Secondary Th1 act	2.2	HUVEC IL-1beta	0.3

Secondary Th2 act	2.8	HUVEC IFN gamma	1.3
Secondary Tr1 act	8.4	HUVEC TNF alpha + IFN gamma	0.0
Secondary Th1 rest	0.4	HUVEC TNF alpha + IL4	0.0
Secondary Th2 rest	3.5	HUVEC IL-11	0.5
Secondary Tr1 rest	0.4	Lung Microvascular EC none	1.0
Primary Th1 act	0.4	Lung Microvascular EC TNFalpha + IL-1beta	0.4
Primary Th2 act	1.2	Microvascular Dermal EC none	2.1
Primary Tr1 act	1.4	Microsvasular Dermal EC TNFalpha + IL-1beta	0.4
Primary Th1 rest	1.6	Bronchial epithelium TNFalpha + IL1beta	12.3
Primary Th2 rest	2.5	Small airway epithelium none	15.6
Primary Tr1 rest	2.4	Small airway epithelium TNFalpha + IL-1beta	**100.0**
CD45RA CD4 lymphocyte act	1.4	Coronery artery SMC rest	3.6
CD45RO CD4 lymphocyte act	1.8	Coronery artery SMC TNFalpha + IL-1beta	0.6
CD8 lymphocyte act	0.5	Astrocytes rest	1.0
Secondary CD8 lymphocyte rest	1.5	Astrocytes TNFalpha + IL-1beta	1.1
Secondary CD8 lymphocyte act	0.0	KU-812 (Basophil) rest	19.1
CD4 lymphocyte none	2.7	KU-812 (Basophil) PMA/ionomycin	48.3
2ry Th1/Th2/Tr1_anti-CD95 CH11	4.2	CCD1106 (Keratinocytes) none	14.6
LAK cells rest	0.0	CCD1106 (Keratinocytes) TNFalpha + IL-1beta	14.3
LAK cells IL-2	1.2	Liver cirrhosis	3.2
LAK cells IL-2+IL-12	1.8	Lupus kidney	0.3
LAK cells IL-2+IFN gamma	2.5	NCI-H292 none	8.5
LAK cells IL-2+ IL-18	1.6	NCI-H292 IL-4	9.3
LAK cells PMA/ionomycin	3.0	NCI-H292 IL-9	13.2
NK Cells IL-2 rest	0.0	NCI-H292 IL-13	4.5
Two Way MLR 3 day	2.4	NCI-H292 IFN gamma	9.7
Two Way MLR 5 day	0.8	HPAEC none	0.4
Two Way MLR 7 day	0.0	HPAEC TNF alpha + IL-1 beta	1.9
PBMC rest	0.8	Lung fibroblast none	0.9
PBMC PWM	4.6	Lung fibroblast TNF alpha + IL-1 beta	0.0
PBMC PHA-L	0.0	Lung fibroblast IL-4	1.0
Ramos (B cell) none	0.8	Lung fibroblast IL-9	1.0
Ramos (B cell) ionomycin	0.8	Lung fibroblast IL-13	1.0
B lymphocytes PWM	0.4	Lung fibroblast IFN gamma	1.1
B lymphocytes CD40L and IL-4	0.7	Dermal fibroblast CCD1070 rest	2.6
EOL-1 dbcAMP	5.6	Dermal fibroblast CCD1070	2.1

		TNF alpha	
EOL-1 dbcAMP PMA/ionomycin	6.3	Dermal fibroblast CCD1070 IL-1 beta	1.9
Dendritic cells none	0.0	Dermal fibroblast IFN gamma	1.1
Dendritic cells LPS	0.3	Dermal fibroblast IL-4	0.6
Dendritic cells anti-CD40	0.9	IBD Colitis 2	0.6
Monocytes rest	0.4	IBD Crohn's	0.4
Monocytes LPS	9.0	Colon	0.1
Macrophages rest	1.2	Lung	0.7
Macrophages LPS	0.1	Thymus	1.3
HUVEC none	0.0	Kidney	2.7
HUVEC starved	0.7		

CNS_neurodegeneration_v1.0 Summary: Ag692 Expression of the CG55023-01 gene is low/undetectable in all samples on this panel (CTs>35). (Data not shown.)

Panel 1 Summary: Ag264/Ag264b Results of three experiments with the CG55023-01 gene show reasonable concordance. The expression of this gene is found to be highest in a sample derived from a prostate cancer cell line (CTs=24-26). In addition, there is substantial expression in a lung cancer cell line. Thus, the expression of this gene could be used to distinguish this prostate cell line sample from the other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, protein therapeutics or antibodies might be of benefit in the treatment of prostate or lung cancer.

Panel 1.2 Summary: Ag692 The expression of the CG55023-01 gene was assessed in two independent runs in this panel with excellent concordance between the results. The expression of this gene is found to be highest in a sample derived from a prostate cancer cell line (CTs=23-24). In addition there is substantial expression in a lung cancer cell line. This expression profile is consistent with the expression seen in Panel 1. Thus, the expression of this gene could be used to distinguish this prostate cell line sample from the other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, protein therapeutics or antibodies might be of benefit in the treatment of prostate or lung cancer.

This gene also shows moderate expression in all CNS regions examined. TGF alpha has numerous roles in the CNS, including regulation of astrocyte reactivity, neuronal differentiation and survival, and protection of motor neurons. Because of its possible neuroprotective effects, this molecule may be of use in the treatment of multiple sclerosis, ALS, Alzheimer's, Parkinson's, or Huntington's diseases, stroke, or brain or spinal cord trauma.

In addition, this gene is moderately expressed in pancreas, adrenal, thyroid, pituitary, skeletal muscle, and adult and fetal liver. Thus, this gene product may be a monoclonal antibody target for the treatment of metabolic and endocrine disease, including obesity and Types 1 and 2 diabetes. Among metabolic tissues, this gene has highest expression in heart
5   (CT values = 27-29), and is 79% identical to mouse epigen protein. Epigen stimulates epithelial cell proliferation (see Panel 4 ref.), suggesting that a monoclonal antibody to this gene product may also be useful for prevention of cardiomyocyte proliferation in diseases of cardiac hypertrophy.

References:

10   Boillee S, Cadusseau J, Coulpier M, Grannec G, Junier MP. Transforming growth factor alpha: a promoter of motoneuron survival of potential biological relevance. J Neurosci 2001 Sep 15;21(18):7079-88

Expression of transforming growth factor alpha (TGFalpha), a member of the epidermal growth factor (EGF) family, is a general response of adult murine motoneurons to
15   genetic and experimental lesions, TGFalpha appearing as an inducer of astrogliosis in these situations. Here we address the possibility that TGFalpha expression is not specific to pathological situations but may participate to the embryonic development of motoneurons. mRNA of TGFalpha and its receptor, the EGF receptor (EGFR), were detected by ribonuclease protection assay in the ventral part of the cervical spinal cord from embryonic
20   day 12 (E12) until adult ages. Reverse transcription-PCR amplification of their transcripts from immunopurified E15 motoneurons, associated with in situ double-immunohistological assays, identified embryonic motoneurons as cellular sources of the TGFalpha-EGFR couple. In vitro, TGFalpha promoted the survival of immunopurified E15 motoneurons in a dose-dependent manner, with a magnitude similar to BDNF neuroprotective effects at equivalent
25   concentrations. In a transgenic mouse expressing a human TGFalpha transgene under the control of the metallothionein 1 promoter, axotomy of the facial nerve provoked significantly less degeneration in the relevant motor pool of 1-week-old mice than in wild-type animals. No protection was observed in neonates, when the transgene exhibits only weak expression levels in the brainstem. In conclusion, our results point to TGFalpha as a physiologically relevant
30   candidate for a neurotrophic role on developing motoneurons. Its expression by the embryonic motoneurons, which also synthesize its receptor, suggests that this chemokine is endowed with the capability to promote motoneuron survival in an autocrine-paracrine manner.

Xian CJ, Zhou XF.Roles of transforming growth factor-alpha and related molecules in the nervous system. Mol Neurobiol 1999 Oct-Dec;20(2-3):157-83

The epidermal growth factor (EGF) family of polypeptides is regulators for tissue development and repair, and is characterized by the fact that their mature forms are

5   proteolytically derived from their integral membrane precursors. This article reviews roles of the prominent members of the EGF family (EGF, transforming growth factor-alpha [TGF-alpha] and heparin-binding EGF [HB-EGF]) and the related neuregulin family in the nerve system. These polypeptides, produced by neurons and glial cells, play an important role in the development of the nervous system, stimulating proliferation, migration, and differentiation of

10   neuronal, glial, and Schwann precursor cells. These peptides are also neurotrophic, enhancing survival and inhibiting apoptosis of post-mitotic neurons, probably acting directly through receptors on neurons, or indirectly via stimulating glial proliferation and glial synthesis of other molecules such as neurotrophic factors. TGF-alpha, EGF, and neuregulins are involved in mediating glial-neuronal and axonal-glial interactions, regulating nerve injury responses,

15   and participating in injury-associated astrocytic gliosis, brain tumors, and other disorders of the nerve system. Although the collective roles of the EGF family (as well as those of the neuregulins) are shown to be essential for the nervous system, redundancy may exist among members of the EGF family.

Junier MP. What role(s) for TGFalpha in the central nervous system? Prog Neurobiol

20   2000 Dec;62(5):443-73

Transforming growth factor alpha (TGFalpha) is a member of the epidermal growth factor (EGF) family with which it shares the same receptor, the EGF receptor (EGFR or erbB1). Identified since 1985 in the central nervous system (CNS), its functions in this organ have started to be determined during the past decade although numerous questions remain

25   unanswered. TGFalpha is widely distributed in the nervous system, both glial and neuronal cells contributing to its synthesis. Although astrocytes appear as its main targets, mediating in part TGFalpha effects on different neuronal populations, results from different studies have raised the possibility for a direct action of this growth factor on neurons. A large array of experimental data have thus pointed to TGFalpha as a multifunctional factor in the CNS. This

30   review is an attempt to present, in a comprehensive manner, the very diverse works performed in vitro and in vivo which have provided evidences for (i) an intervention of TGFalpha in the control of developmental events such as neural progenitors proliferation/cell fate choice, neuronal survival/differentiation, and neuronal control of female puberty onset, (ii) its role as a potent regulator of astroglial metabolism including astrocytic reactivity, (iii) its

neuroprotective potential, and (iv) its participation to neuropathological processes as exemplified by astroglial neoplasia. In addition, informations regarding the complex modes of TGFalpha action at the molecular level are provided, and its place within the large EGF family is precised with regard to the potential interactions and substitutions which may take place

5   between TGFalpha and its kindred.

Panel 2D Summary: Ag264/692 The expression of the CG55023-01 gene was assessed in two independent runs on panel 2D using two different probe/primer pairs. The expression of this gene appears to be highest in samples derived from lung cancer tissue (CTs=28-30). In addition, there is substantial expression in samples derived from two breast

10   cancers, bladder cancer and a sample of normal ovarian tissue. Thus, the expression of this gene could be used to distinguish these lung cancer samples from other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics may be of benefit to the treatment of lung cancer, breast cancer or bladder cancer.

15   **Panel 4D Summary:** Ag692 The CG55023-01, a TGF-alpha-like Epigen protein homolog, is most highly expressed in small airway epithelium activated with TNFalpha + IL-1beta (CT=28.71) and in KU-812 basophil cells activated with phorbol ester and ionomycin (CT=29.76). Epigne has been shown to stimulate the growth of epithelial cells. Therefore, antibodies that block the action of the CG55023-01 gene product may be useful as therapeutics

20   to reduce or eliminate the symptoms in patients with asthma, emphysema, and allergy.

References:

Strachan L, Murison JG, Prestidge RL, Sleeman MA, Watson JD, Kumble KD. Cloning and biological activity of epigen, a novel member of the epidermal growth factor superfamily. J Biol Chem. 2001 May 25;276(21):18265-71.

25   High throughput sequencing of a mouse keratinocyte library was used to identify an expressed sequence tag with homology to the epidermal growth factor (EGF) family of growth factors. We have named the protein encoded by this expressed sequence tag Epigen, for epithelial mitogen. Epigen encodes a protein of 152 amino acids that contains features characteristic of the EGF superfamily. Two hydrophobic regions, corresponding to a putative

30   signal sequence and transmembrane domain, flank a core of amino acids encompassing six cysteine residues and two putative N-linked glycosylation sites. Epigen shows 24-37% identity to members of the EGF superfamily including EGF, transforming growth factor alpha, and Epiregulin. Northern blotting of several adult mouse tissues indicated that Epigen was present

in testis, heart, and liver. Recombinant Epigen was synthesized in Escherichia coli and refolded, and its biological activity was compared with that of EGF and transforming growth factor alpha in several assays. In epithelial cells, Epigen stimulated the phosphorylation of c-erbB-1 and mitogen-activated protein kinases and also activated a reporter gene containing enhancer sequences present in the c-fos promoter. Epigen also stimulated the proliferation of HaCaT cells, and this proliferation was blocked by an antibody to the extracellular domain of the receptor tyrosine kinase c-erbB-1. Thus, Epigen is the newest member of the EGF superfamily and, with its ability to promote the growth of epithelial cells, may constitute a novel molecular target for wound-healing therapy.

PMID: 11278323

SEC1 (CG55688-01)

Expression of gene CG55688-01 was assessed using the primer-probe set Ag1148, described in Table 23A. Results of the RTQ-PCR runs are shown in Tables 23B, 23C, 23D, 23E, 23F, 23G and 23H.

Table 23A. Probe Name Ag1148

Primers	Sequences	Length	Start Position
Forward	5'-gtgtctgtgagaggcagctatc-3' (SEQ ID NO:153)	22	1683
Probe	TET-5'-tgcactctaaactgcaaacagaaatcagg-3'-TAMRA (SEQ ID NO:154)	29	1705
Reverse	5'-ccccaaaagctacattttgata-3' (SEQ ID NO:155)	22	1758

Table 23B. General_screening_panel_v1.5

Tissue Name	Rel. Exp.(%) Ag1148, Run 230220165	Tissue Name	Rel. Exp.(%) Ag1148, Run 230220165
Adipose	2.8	Renal ca. TK-10	23.7
Melanoma* Hs688(A).T	100.0	Bladder	1.8
Melanoma* Hs688(B).T	57.0	Gastric ca. (liver met.) NCI-N87	2.0
Melanoma* M14	0.0	Gastric ca. KATO III	0.5
Melanoma* LOXIMVI	10.0	Colon ca. SW-948	0.0
Melanoma* SK-MEL-5	0.0	Colon ca. SW480	3.4
Squamous cell carcinoma SCC-4	3.1	Colon ca.* (SW480 met) SW620	0.0
Testis Pool	1.6	Colon ca. HT29	0.0
Prostate ca.* (bone met) PC-3	1.6	Colon ca. HCT-116	0.6
Prostate Pool	0.6	Colon ca. CaCo-2	0.5
Placenta	1.0	Colon cancer tissue	8.4
Uterus Pool	0.0	Colon ca. SW1116	0.0
Ovarian ca. OVCAR-3	2.9	Colon ca. Colo-205	0.0
Ovarian ca. SK-OV-3	6.7	Colon ca. SW-48	0.0
Ovarian ca. OVCAR-4	46.3	Colon Pool	14.9

Ovarian ca. OVCAR-5	3.6	Small Intestine Pool	2.6
Ovarian ca. IGROV-1	5.7	Stomach Pool	4.7
Ovarian ca. OVCAR-8	6.2	Bone Marrow Pool	1.1
Ovary	6.3	Fetal Heart	1.9
Breast ca. MCF-7	0.0	Heart Pool	3.6
Breast ca. MDA-MB-231	21.2	Lymph Node Pool	5.8
Breast ca. BT 549	32.8	Fetal Skeletal Muscle	2.0
Breast ca. T47D	1.6	Skeletal Muscle Pool	3.0
Breast ca. MDA-N	0.0	Spleen Pool	7.4
Breast Pool	12.9	Thymus Pool	8.7
Trachea	4.6	CNS cancer (glio/astro) U87-MG	0.0
Lung	6.5	CNS cancer (glio/astro) U-118-MG	5.8
Fetal Lung	23.7	CNS cancer (neuro;met) SK-N-AS	0.0
Lung ca. NCI-N417	0.0	CNS cancer (astro) SF-539	6.4
Lung ca. LX-1	0.0	CNS cancer (astro) SNB-75	37.6
Lung ca. NCI-H146	0.0	CNS cancer (glio) SNB-19	0.0
Lung ca. SHP-77	0.0	CNS cancer (glio) SF-295	10.4
Lung ca. A549	0.7	Brain (Amygdala) Pool	0.0
Lung ca. NCI-H526	0.0	Brain (cerebellum)	0.0
Lung ca. NCI-H23	1.6	Brain (fetal)	0.0
Lung ca. NCI-H460	0.0	Brain (Hippocampus) Pool	0.0
Lung ca. HOP-62	0.0	Cerebral Cortex Pool	0.0
Lung ca. NCI-H522	8.5	Brain (Substantia nigra) Pool	0.0
Liver	0.0	Brain (Thalamus) Pool	0.0
Fetal Liver	0.0	Brain (whole)	0.0
Liver ca. HepG2	0.0	Spinal Cord Pool	0.0
Kidney Pool	49.3	Adrenal Gland	1.0
Fetal Kidney	1.5	Pituitary gland Pool	0.5
Renal ca. 786-0	15.0	Salivary Gland	0.0
Renal ca. A498	2.5	Thyroid (female)	3.5
Renal ca. ACHN	14.4	Pancreatic ca. CAPAN2	0.6
Renal ca. UO-31	17.7	Pancreas Pool	18.7

Table 23C. Panel 1.2

Tissue Name	Rel. Exp.(%) Ag1148, Run 126901413	Rel. Exp.(%) Ag1148, Run 127126116	Tissue Name	Rel. Exp.(%) Ag1148, Run 126901413	Rel. Exp.(%) Ag1148, Run 127126116
Endothelial cells	11.0	19.3	Renal ca. 786-0	16.4	4.9
Heart (Fetal)	49.7	**100.0**	Renal ca. A498	0.0	0.0
Pancreas	0.9	0.0	Renal ca. RXF 393	30.6	33.9
Pancreatic ca. CAPAN 2	0.0	0.0	Renal ca. ACHN	27.2	21.9
Adrenal Gland	76.8	63.3	Renal ca. UO-31	17.4	0.1
Thyroid	6.1	1.0	Renal ca. TK-10	22.5	3.9
Salivary gland	19.3	3.1	Liver	39.2	58.6

Pituitary gland	3.4	0.1	Liver (fetal)	12.9	16.4
Brain (fetal)	0.8	1.0	Liver ca. (hepatoblast) HepG2	0.5	0.0
Brain (whole)	0.2	0.0	Lung	59.9	92.0
Brain (amygdala)	0.1	0.0	Lung (fetal)	9.5	7.2
Brain (cerebellum)	0.0	0.0	Lung ca. (small cell) LX-1	0.0	0.0
Brain (hippocampus)	0.6	0.5	Lung ca. (small cell) NCI-H69	0.0	0.0
Brain (thalamus)	0.0	0.0	Lung ca. (s.cell var.) SHP-77	0.0	0.0
Cerebral Cortex	0.1	0.0	Lung ca. (large cell)NCI-H460	3.7	3.0
Spinal cord	1.0	0.0	Lung ca. (non-sm. cell) A549	0.0	0.0
glio/astro U87-MG	0.2	0.0	Lung ca. (non-s.cell) NCI-H23	0.9	0.0
glio/astro U-118-MG	10.7	8.2	Lung ca. (non-s.cell) HOP-62	1.5	0.0
astrocytoma SW1783	17.1	17.7	Lung ca. (non-s.cl) NCI-H522	53.2	24.0
neuro*; met SK-N-AS	0.0	0.0	Lung ca. (squam.) SW 900	0.2	0.0
astrocytoma SF-539	8.8	4.2	Lung ca. (squam.) NCI-H596	0.0	0.0
astrocytoma SNB-75	2.0	0.5	Mammary gland	45.1	19.3
glioma SNB-19	9.2	5.3	Breast ca.* (pl.ef) MCF-7	0.0	0.0
glioma U251	11.5	1.6	Breast ca.* (pl.ef) MDA-MB-231	14.9	2.2
glioma SF-295	4.4	0.2	Breast ca.* (pl. ef) T47D	0.0	0.0
Heart	**100.0**	87.7	Breast ca. BT-549	16.5	13.3
Skeletal Muscle	10.7	5.2	Breast ca. MDA-N	0.0	0.0
Bone marrow	1.1	1.6	Ovary	22.4	28.1
Thymus	0.3	0.4	Ovarian ca. OVCAR-3	0.0	0.1
Spleen	3.3	1.0	Ovarian ca. OVCAR-4	56.3	57.8
Lymph node	30.8	48.0	Ovarian ca. OVCAR-5	0.0	0.0
Colorectal Tissue	0.2	0.0	Ovarian ca. OVCAR-8	25.0	2.7
Stomach	0.5	0.0	Ovarian ca. IGROV-1	3.2	0.0
Small intestine	4.0	1.4	Ovarian ca. (ascites) SK-OV-3	0.2	0.0
Colon ca. SW480	0.0	0.0	Uterus	12.3	15.8

Colon ca.* SW620 (SW480 met)	0.0	0.0	Placenta	32.1	28.1
Colon ca. HT29	0.0	0.0	Prostate	8.8	0.8
Colon ca. HCT-116	0.0	0.0	Prostate ca.* (bone met) PC-3	0.0	0.0
Colon ca. CaCo-2	0.0	0.0	Testis	0.9	0.0
Colon ca. Tissue (ODO3866)	4.5	3.8	Melanoma Hs688(A).T	73.2	45.7
Colon ca. HCC-2998	0.0	0.0	Melanoma* (met) Hs688(B).T	35.6	13.1
Gastric ca.* (liver met) NCI-N87	0.1	0.0	Melanoma UACC-62	0.0	0.0
Bladder	15.3	23.5	Melanoma M14	0.0	0.0
Trachea	8.0	10.9	Melanoma LOX IMVI	17.4	2.9
Kidney	21.2	3.2	Melanoma* (met) SK-MEL-5	0.0	0.0
Kidney (fetal)	29.7	29.9			

Table 23D. Panel 1.3D

Tissue Name	Rel. Exp.(%) Ag1148, Run 151759893	Tissue Name	Rel. Exp.(%) Ag1148, Run 151759893
Liver adenocarcinoma	4.5	Kidney (fetal)	9.9
Pancreas	1.8	Renal ca. 786-0	13.0
Pancreatic ca. CAPAN 2	0.6	Renal ca. A498	11.1
Adrenal gland	6.3	Renal ca. RXF 393	20.2
Thyroid	11.2	Renal ca. ACHN	17.2
Salivary gland	1.5	Renal ca. UO-31	26.1
Pituitary gland	1.3	Renal ca. TK-10	10.2
Brain (fetal)	0.8	Liver	1.0
Brain (whole)	1.4	Liver (fetal)	8.0
Brain (amygdala)	0.8	Liver ca. (hepatoblast) HepG2	0.9
Brain (cerebellum)	0.1	Lung	45.4
Brain (hippocampus)	5.9	Lung (fetal)	34.9
Brain (substantia nigra)	1.7	Lung ca. (small cell) LX-1	0.1
Brain (thalamus)	1.2	Lung ca. (small cell) NCI-H69	0.0
Cerebral Cortex	1.5	Lung ca. (s.cell var.) SHP-77	0.0
Spinal cord	2.0	Lung ca. (large cell)NCI-H460	0.2
glio/astro U87-MG	0.7	Lung ca. (non-sm. cell) A549	0.2
glio/astro U-118-MG	19.6	Lung ca. (non-s.cell) NCI-H23	2.8
astrocytoma SW1783	21.8	Lung ca. (non-s.cell) HOP-62	1.9
neuro*; met SK-N-AS	0.8	Lung ca. (non-s.cl) NCI-H522	12.2
astrocytoma SF-539	16.3	Lung ca. (squam.) SW 900	2.7

astrocytoma SNB-75	11.3	Lung ca. (squam.) NCI-H596	0.0
glioma SNB-19	8.4	Mammary gland	45.4
glioma U251	10.2	Breast ca.* (pl.ef) MCF-7	0.1
glioma SF-295	5.6	Breast ca.* (pl.ef) MDA-MB-231	16.7
Heart (fetal)	28.9	Breast ca.* (pl.ef) T47D	1.3
Heart	9.5	Breast ca. BT-549	11.1
Skeletal muscle (fetal)	14.1	Breast ca. MDA-N	0.3
Skeletal muscle	3.3	Ovary	16.3
Bone marrow	1.7	Ovarian ca. OVCAR-3	1.6
Thymus	1.2	Ovarian ca. OVCAR-4	12.7
Spleen	12.6	Ovarian ca. OVCAR-5	1.9
Lymph node	21.3	Ovarian ca. OVCAR-8	12.6
Colorectal	8.6	Ovarian ca. IGROV-1	1.8
Stomach	5.2	Ovarian ca.* (ascites) SK-OV-3	3.5
Small intestine	9.9	Uterus	11.0
Colon ca. SW480	2.6	Placenta	9.0
Colon ca.* SW620(SW480 met)	0.1	Prostate	5.7
Colon ca. HT29	0.1	Prostate ca.* (bone met)PC-3	1.9
Colon ca. HCT-116	1.0	Testis	3.8
Colon ca. CaCo-2	0.7	Melanoma Hs688(A).T	**100.0**
Colon ca. tissue(ODO3866)	9.8	Melanoma* (met) Hs688(B).T	88.3
Colon ca. HCC-2998	0.6	Melanoma UACC-62	0.2
Gastric ca.* (liver met) NCI-N87	3.0	Melanoma M14	0.1
Bladder	2.5	Melanoma LOX IMVI	3.1
Trachea	17.9	Melanoma* (met) SK-MEL-5	0.1
Kidney	1.8	Adipose	45.4

Table 23E. Panel 2D

Tissue Name	Rel. Exp.(%) Ag1148, Run 145375638	Rel. Exp.(%) Ag1148, Run 147104767	Tissue Name	Rel. Exp.(%) Ag1148, Run 145375638	Rel. Exp.(%) Ag1148, Run 147104767
Normal Colon	5.0	15.7	Kidney Margin 8120608	6.3	6.6
CC Well to Mod Diff (ODO3866)	3.9	14.6	Kidney Cancer 8120613	0.6	0.7
CC Margin (ODO3866)	9.0	23.7	Kidney Margin 8120614	1.1	4.0
CC Gr.2 rectosigmoid (ODO3868)	0.7	1.7	Kidney Cancer 9010320	4.3	14.6
CC Margin (ODO3868)	3.3	5.6	Kidney Margin 9010321	4.7	6.7
CC Mod Diff (ODO3920)	0.4	1.0	Normal Uterus	33.2	25.5

CC Margin (ODO3920)	3.0	5.9	Uterus Cancer 064011	72.7	44.1
CC Gr.2 ascend colon (ODO3921)	67.4	14.3	Normal Thyroid	8.6	11.2
CC Margin (ODO3921)	3.5	11.3	Thyroid Cancer 064010	8.4	5.0
CC from Partial Hepatectomy (ODO4309) Mets	2.3	6.0	Thyroid Cancer A302152	5.5	4.9
Liver Margin (ODO4309)	0.9	3.5	Thyroid Margin A302153	40.1	35.4
Colon mets to lung (OD04451-01)	4.2	3.9	Normal Breast	25.3	23.2
Lung Margin (OD04451-02)	1.7	3.5	Breast Cancer (OD04566)	4.2	2.5
Normal Prostate 6546-1	4.9	5.1	Breast Cancer (OD04590-01)	1.5	3.8
Prostate Cancer (OD04410)	21.9	29.9	Breast Cancer Mets (OD04590-03)	16.4	9.7
Prostate Margin (OD04410)	39.2	30.8	Breast Cancer Metastasis (OD04655-05)	3.1	2.3
Prostate Cancer (OD04720-01)	9.7	8.6	Breast Cancer 064006	5.0	5.3
Prostate Margin (OD04720-02)	49.3	44.4	Breast Cancer 1024	1.6	5.1
Normal Lung 061010	19.3	21.5	Breast Cancer 9100266	5.2	3.9
Lung Met to Muscle (ODO4286)	2.8	2.6	Breast Margin 9100265	6.7	5.1
Muscle Margin (ODO4286)	12.3	8.0	Breast Cancer A209073	8.8	5.6
Lung Malignant Cancer (OD03126)	10.0	1.0	Breast Margin A2090734	1.0	1.8
Lung Margin (OD03126)	17.6	32.3	Normal Liver	0.6	0.6
Lung Cancer (OD04404)	4.7	11.7	Liver Cancer 064003	0.2	0.8
Lung Margin (OD04404)	3.0	7.2	Liver Cancer 1025	1.6	4.7
Lung Cancer (OD04565)	4.0	2.8	Liver Cancer 1026	1.4	3.6
Lung Margin (OD04565)	11.8	4.7	Liver Cancer 6004-T	2.2	4.1
Lung Cancer (OD04237-01)	5.1	5.1	Liver Tissue 6004-N	0.3	2.1
Lung Margin (OD04237-02)	7.2	21.6	Liver Cancer 6005-T	2.0	4.4
Ocular Mel Met to Liver (ODO4310)	1.2	0.7	Liver Tissue 6005-N	0.8	1.8
Liver Margin (ODO4310)	6.9	5.2	Normal Bladder	6.2	8.9
Melanoma Mets to Lung (OD04321)	3.0	2.9	Bladder Cancer 1023	3.8	4.6

Lung Margin (OD04321)	33.2	37.4	Bladder Cancer A302173	0.8	2.4
Normal Kidney	11.2	21.5	Bladder Cancer (OD04718-01)	6.2	9.7
Kidney Ca, Nuclear grade 2 (OD04338)	11.8	17.2	Bladder Normal Adjacent (OD04718-03)	50.0	**100.0**
Kidney Margin (OD04338)	17.8	34.6	Normal Ovary	1.6	7.5
Kidney Ca Nuclear grade 1/2 (OD04339)	2.6	5.2	Ovarian Cancer 064008	26.1	69.3
Kidney Margin (OD04339)	15.8	15.4	Ovarian Cancer (OD04768-07)	7.7	18.0
Kidney Ca, Clear cell type (OD04340)	**100.0**	59.0	Ovary Margin (OD04768-08)	75.8	62.0
Kidney Margin (OD04340)	36.1	57.0	Normal Stomach	2.6	8.0
Kidney Ca, Nuclear grade 3 (OD04348)	2.6	2.0	Gastric Cancer 9060358	0.5	1.5
Kidney Margin (OD04348)	25.3	14.5	Stomach Margin 9060359	2.5	7.0
Kidney Cancer (OD04622-01)	32.8	15.5	Gastric Cancer 9060395	1.5	6.0
Kidney Margin (OD04622-03)	6.2	3.7	Stomach Margin 9060394	2.1	8.7
Kidney Cancer (OD04450-01)	12.5	9.1	Gastric Cancer 9060397	4.4	16.7
Kidney Margin (OD04450-03)	19.5	14.8	Stomach Margin 9060396	0.6	1.9
Kidney Cancer 8120607	3.8	2.9	Gastric Cancer 064005	2.0	7.0

Table 23F. Panel 3D

Tissue Name	Rel. Exp.(%) Ag1148, Run 163476715	Tissue Name	Rel. Exp.(%) Ag1148, Run 163476715
Daoy- Medulloblastoma	15.3	Ca Ski- Cervical epidermoid carcinoma (metastasis)	52.9
TE671- Medulloblastoma	0.0	ES-2- Ovarian clear cell carcinoma	26.8
D283 Med- Medulloblastoma	1.5	Ramos- Stimulated with PMA/ionomycin 6h	0.0
PFSK-1- Primitive Neuroectodermal	3.4	Ramos- Stimulated with PMA/ionomycin 14h	0.1
XF-498- CNS	22.1	MEG-01- Chronic myelogenous leukemia (megokaryoblast)	0.9
SNB-78- Glioma	25.2	Raji- Burkitt's lymphoma	0.1
SF-268- Glioblastoma	60.3	Daudi- Burkitt's lymphoma	0.2
T98G- Glioblastoma	19.2	U266- B-cell plasmacytoma	0.0
SK-N-SH- Neuroblastoma (metastasis)	13.0	CA46- Burkitt's lymphoma	0.0
SF-295- Glioblastoma	3.5	RL- non-Hodgkin's B-cell lymphoma	0.0
Cerebellum	0.3	JM1- pre-B-cell lymphoma	0.0

Cerebellum	0.1	Jurkat- T cell leukemia	0.0
NCI-H292- Mucoepidermoid lung carcinoma	**100.0**	TF-1- Erythroleukemia	0.3
DMS-114- Small cell lung cancer	2.7	HUT 78- T-cell lymphoma	0.0
DMS-79- Small cell lung cancer	0.2	U937- Histiocytic lymphoma	0.0
NCI-H146- Small cell lung cancer	0.0	KU-812- Myelogenous leukemia	0.2
NCI-H526- Small cell lung cancer	0.0	769-P- Clear cell renal carcinoma	17.7
NCI-N417- Small cell lung cancer	0.0	Caki-2- Clear cell renal carcinoma	1.7
NCI-H82- Small cell lung cancer	0.1	SW 839- Clear cell renal carcinoma	71.7
NCI-H157- Squamous cell lung cancer (metastasis)	42.0	G401- Wilms' tumor	3.0
NCI-H1155- Large cell lung cancer	0.5	Hs766T- Pancreatic carcinoma (LN metastasis)	15.4
NCI-H1299- Large cell lung cancer	23.5	CAPAN-1- Pancreatic adenocarcinoma (liver metastasis)	6.9
NCI-H727- Lung carcinoid	0.3	SU86.86- Pancreatic carcinoma (liver metastasis)	14.6
NCI-UMC-11- Lung carcinoid	0.1	BxPC-3- Pancreatic adenocarcinoma	3.6
LX-1- Small cell lung cancer	0.0	HPAC- Pancreatic adenocarcinoma	4.0
Colo-205- Colon cancer	0.0	MIA PaCa-2- Pancreatic carcinoma	3.4
KM12- Colon cancer	0.9	CFPAC-1- Pancreatic ductal adenocarcinoma	28.3
KM20L2- Colon cancer	0.2	PANC-1- Pancreatic epithelioid ductal carcinoma	16.2
NCI-H716- Colon cancer	1.0	T24- Bladder carcinma (transitional cell)	12.1
SW-48- Colon adenocarcinoma	0.0	5637- Bladder carcinoma	3.1
SW1116- Colon adenocarcinoma	0.5	HT-1197- Bladder carcinoma	14.6
LS 174T- Colon adenocarcinoma	0.8	UM-UC-3- Bladder carcinma (transitional cell)	4.6
SW-948- Colon adenocarcinoma	0.0	A204- Rhabdomyosarcoma	0.7
SW-480- Colon adenocarcinoma	0.0	HT-1080- Fibrosarcoma	15.8
NCI-SNU-5- Gastric carcinoma	3.5	MG-63- Osteosarcoma	53.2
KATO III- Gastric carcinoma	0.7	SK-LMS-1- Leiomyosarcoma (vulva)	55.5
NCI-SNU-16- Gastric carcinoma	6.3	SJRH30- Rhabdomyosarcoma (met to bone marrow)	1.7
NCI-SNU-1- Gastric carcinoma	0.4	A431- Epidermoid carcinoma	1.6
RF-1- Gastric adenocarcinoma	0.0	WM266-4- Melanoma	0.5
RF-48- Gastric	0.0	DU 145- Prostate carcinoma (brain	1.8

adenocarcinoma		metastasis)	
MKN-45- Gastric carcinoma	1.6	MDA-MB-468- Breast adenocarcinoma	1.8
NCI-N87- Gastric carcinoma	1.0	SCC-4- Squamous cell carcinoma of tongue	1.3
OVCAR-5- Ovarian carcinoma	0.9	SCC-9- Squamous cell carcinoma of tongue	1.0
RL95-2- Uterine carcinoma	1.4	SCC-15- Squamous cell carcinoma of tongue	1.3
HelaS3- Cervical adenocarcinoma	1.6	CAL 27- Squamous cell carcinoma of tongue	20.7

Table 23G. Panel 4D

Tissue Name	Rel. Exp.(%) Ag1148, Run 145386435	Tissue Name	Rel. Exp.(%) Ag1148, Run 145386435
Secondary Th1 act	0.0	HUVEC IL-1beta	17.7
Secondary Th2 act	0.0	HUVEC IFN gamma	40.3
Secondary Tr1 act	0.0	HUVEC TNF alpha + IFN gamma	30.1
Secondary Th1 rest	0.0	HUVEC TNF alpha + IL4	42.6
Secondary Th2 rest	0.0	HUVEC IL-11	19.8
Secondary Tr1 rest	0.0	Lung Microvascular EC none	63.7
Primary Th1 act	0.1	Lung Microvascular EC TNFalpha + IL-1beta	46.7
Primary Th2 act	0.3	Microvascular Dermal EC none	93.3
Primary Tr1 act	0.5	Microsvasular Dermal EC TNFalpha + IL-1beta	71.7
Primary Th1 rest	0.5	Bronchial epithelium TNFalpha + IL1beta	16.7
Primary Th2 rest	0.4	Small airway epithelium none	5.3
Primary Tr1 rest	0.1	Small airway epithelium TNFalpha + IL-1beta	29.5
CD45RA CD4 lymphocyte act	39.0	Coronery artery SMC rest	46.0
CD45RO CD4 lymphocyte act	0.1	Coronery artery SMC TNFalpha + IL-1beta	48.0
CD8 lymphocyte act	0.1	Astrocytes rest	13.0
Secondary CD8 lymphocyte rest	0.0	Astrocytes TNFalpha + IL-1beta	28.7
Secondary CD8 lymphocyte act	0.0	KU-812 (Basophil) rest	0.2
CD4 lymphocyte none	0.0	KU-812 (Basophil) PMA/ionomycin	0.7
2ry Th1/Th2/Tr1_anti-CD95 CH11	0.3	CCD1106 (Keratinocytes) none	4.5
LAK cells rest	0.0	CCD1106 (Keratinocytes) TNFalpha + IL-1beta	12.7
LAK cells IL-2	0.1	Liver cirrhosis	7.1
LAK cells IL-2+IL-12	0.3	Lupus kidney	2.5
LAK cells IL-2+IFN gamma	0.4	NCI-H292 none	23.7
LAK cells IL-2+ IL-18	0.0	NCI-H292 IL-4	12.3
LAK cells	0.3	NCI-H292 IL-9	11.7

PMA/ionomycin			
NK Cells IL-2 rest	0.0	NCI-H292 IL-13	12.2
Two Way MLR 3 day	0.1	NCI-H292 IFN gamma	13.5
Two Way MLR 5 day	0.2	HPAEC none	42.3
Two Way MLR 7 day	0.0	HPAEC TNF alpha + IL-1 beta	51.8
PBMC rest	0.0	Lung fibroblast none	24.8
PBMC PWM	0.4	Lung fibroblast TNF alpha + IL-1 beta	6.7
PBMC PHA-L	0.3	Lung fibroblast IL-4	41.8
Ramos (B cell) none	0.7	Lung fibroblast IL-9	32.8
Ramos (B cell) ionomycin	1.4	Lung fibroblast IL-13	89.5
B lymphocytes PWM	1.0	Lung fibroblast IFN gamma	69.3
B lymphocytes CD40L and IL-4	0.6	Dermal fibroblast CCD1070 rest	**100.0**
EOL-1 dbcAMP	0.3	Dermal fibroblast CCD1070 TNF alpha	77.9
EOL-1 dbcAMP PMA/ionomycin	0.4	Dermal fibroblast CCD1070 IL-1 beta	95.3
Dendritic cells none	0.0	Dermal fibroblast IFN gamma	5.8
Dendritic cells LPS	0.0	Dermal fibroblast IL-4	8.0
Dendritic cells anti-CD40	0.1	IBD Colitis 2	3.0
Monocytes rest	0.1	IBD Crohn's	5.3
Monocytes LPS	0.1	Colon	2.5
Macrophages rest	0.2	Lung	11.4
Macrophages LPS	0.0	Thymus	9.1
HUVEC none	49.3	Kidney	2.6
HUVEC starved	41.2		

Table 23H. Panel 5 Islet

Tissue Name	Rel. Exp.(%) Ag1148, Run 233070519	Tissue Name	Rel. Exp.(%) Ag1148, Run 233070519
97457_Patient-02go_adipose	6.2	94709_Donor 2 AM - A_adipose	17.1
97476_Patient-07sk_skeletal muscle	77.4	94710_Donor 2 AM - B_adipose	3.0
97477_Patient-07ut_uterus	14.7	94711_Donor 2 AM - C_adipose	7.6
97478_Patient-07pl_placenta	7.1	94712_Donor 2 AD - A_adipose	35.8
99167_Bayer Patient 1	18.6	94713_Donor 2 AD - B_adipose	37.4
97482_Patient-08ut_uterus	5.8	94714_Donor 2 AD - C_adipose	44.8
97483_Patient-08pl_placenta	3.3	94742_Donor 3 U - A_Mesenchymal Stem Cells	5.1
97486_Patient-09sk_skeletal muscle	11.7	94743_Donor 3 U - B_Mesenchymal Stem Cells	15.0
97487_Patient-09ut_uterus	12.4	94730_Donor 3 AM - A_adipose	15.2
97488_Patient-09pl_placenta	1.9	94731_Donor 3 AM - B_adipose	10.8
97492_Patient-10ut_uterus	15.7	94732_Donor 3 AM - C_adipose	11.7
97493_Patient-10pl_placenta	3.5	94733_Donor 3 AD - A_adipose	**100.0**
97495_Patient-	41.2	94734_Donor 3 AD - B_adipose	11.7

11go_adipose			
97496_Patient-11sk_skeletal muscle	8.7	94735_Donor 3 AD - C_adipose	44.8
97497_Patient-11ut_uterus	12.8	77138_Liver_HepG2untreated	15.0
97498_Patient-11pl_placenta	3.1	73556_Heart_Cardiac stromal cells (primary)	29.9
97500_Patient-12go_adipose	72.2	81735_Small Intestine	7.7
97501_Patient-12sk_skeletal muscle	31.6	72409_Kidney_Proximal Convoluted Tubule	5.5
97502_Patient-12ut_uterus	12.2	82685_Small intestine_Duodenum	5.0
97503_Patient-12pl_placenta	4.5	90650_Adrenal_Adrenocortical adenoma	2.4
94721_Donor 2 U - A_Mesenchymal Stem Cells	36.1	72410_Kidney_HRCE	46.0
94722_Donor 2 U - B_Mesenchymal Stem Cells	12.2	72411_Kidney_HRE	21.6
94723_Donor 2 U - C_Mesenchymal Stem Cells	47.3	73139_Uterus_Uterine smooth muscle cells	7.3

General_screening_panel_v1.5 Summary: Ag1148 The expression of the CG55688-01 gene appears to be highest in a sample derived from a melanoma cell line (Hs.688(A).T)(CT=32.2). Overall, significant expression is predominantly seen in cancer cell lines, including a related melanoma cell line (Hs.688(B).T), as well as a cluster of renal, ovarian and breast cancer cell lines. Thus, the expression of this gene could be used to distinguish Hs.688(A).T cells from the other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, protein therapeutics or antibodies might be of benefit in the treatment of melanoma, renal cancer, breast cancer or ovarian cancer.

There is also significant expression in kidney (CT=33.2) when compared to expression in fetal kidney (CT=38.2). Thus, expression of this gene could be used to differentiate between adult and fetal kidney.

Panel 1.2 Summary: Ag1148 Two experiments with the same probe and primer set show highest expression of the CG55688-01 gene in fetal and adult heart (CTs=25). This gene has moderate to high expression in other metabolic tissues, including pancreas, adrenal, thyroid, pituitary, skeletal muscle and adult and fetal liver. This gene product belongs to the insulin-like growth factor binding protein family and, by homology, may play myriad roles in metabolic regulation. Therefore, this gene product may be a monoclonal antibody target for the treatment of metabolic and endocrine diseases, including obesity and Types 1 and 2 diabetes.

This panel shows that the expression of this gene within the CNS is the highest in the hippocampus. The hippocampus is a region of the brain critical for the formation of long-term memories. Please see panel 1.3d for a discussion of utility of this gene in the central nervous system.

5   There is also significant expression in lung (CTs=25-27) when compared to expression in fetal lung (CTs=29-30). Thus, expression of this gene could be used to differentiate between adult and fetal lung.

Panel 1.3D Summary: Ag1148 The expression of the CG55688-01 gene appears to be highest in a sample derived from a melanoma cell line (Hs.688(A).T) (CT=27). In addition,
10   there appears to be substantial expression in a related melanoma cell line (Hs.688(B).T) as well as a cluster of brain cancer cell lines and renal cancer cell lines. This expression is consistent with expression in the previous panels. Thus, the expression of this gene could be used to distinguish Hs.688(A).T cells from the other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, protein
15   therapeutics or antibodies might be of benefit in the treatment of melanoma, renal cancer or brain cancer.

This panel shows significant expression of this gene in metabolic tissues, confirming expression seen in Panel 1.2. Please see that panel for discussion of the utility of this gene in metabolic disease.

20   In addition, this gene is expressed at low levels in several brain regions including hippocampus, cortex, substantia nigra, thalamus, amygdala, and the fetal brain. Cry61 is an immediate early gene that has been implicated in memory formation and synaptic plasticity. It has also been shown to be upregulated during the development of the hippocampus, which is a critical brain region for the formation of long-term memory. Based on its homology to Cry61
25   and its preferential expression in the hippocampus, this gene is therefore an excellent drug target for the treatment of dementia (Alzheimer's, vascular, etc) or for memory enhancement.

References:

Albrecht C, von Der Kammer H, Mayhaus M, Klaudiny J, Schweizer M, Nitsch RM. Muscarinic acetylcholine receptors induce the expression of the immediate early growth
30   regulatory gene CYR61. J Biol Chem 2000 Sep 15;275(37):28929-36

In brain, muscarinic acetylcholine receptors (mAChRs) modulate neuronal functions including long term potentiation and synaptic plasticity in neuronal circuits that are involved in learning and memory formation. To identify mAChR-inducible genes, we used a

differential display approach and found that mAChRs rapidly induced transcription of the immediate early gene CYR61 in HEK 293 cells with a maximum expression after 1 h of receptor stimulation. CYR61 is a member of the emerging CCN gene family that includes CYR61/CEF10, CTGF/FISP-12, and NOV; these encode secretory growth regulatory proteins

5   with distinct functions in cell proliferation, migration, adhesion, and survival. We found that CYR61, CTGF, and NOV were expressed throughout the human central nervous system. Stimulation of mAChRs induced CYR61 expression in primary neurons and rat brain where CYR61 mRNA was detected in cortical layers V and VI and in thalamic nuclei. In contrast, CTGF and NOV expression was not altered by mAChRs neither in neuronal tissue culture nor

10   rat brain. Receptor subtype analyses demonstrated that m1 and m3 mAChR subtypes strongly induced CYR61 expression, whereas m2 and m4 mAChRs had only subtle effects. Increased CYR61 expression was coupled to mAChRs by both protein kinase C and elevations of intracellular Ca(2+). Our results establish that CYR61 expression in mammalian brain is under the control of cholinergic neurotransmission; it may thus be involved in cholinergic regulation

15   of synaptic plasticity.

       Chung KC, Ahn YS. Expression of immediate early gene cyr61 during the differentiation of immortalized embryonic hippocampal neuronal cells. Neurosci Lett 1998 Oct 23;255(3):155-8

       Growth factor-mediated signal transduction is a process that is of fundamental

20   importance in understanding cellular growth and differentiation. In order to elucidate the signaling pathways leading to neuronal differentiation, we have tried to identify intermediates that are selectively induced in the differentiation of immortalized neuronal hippocampal cell line H19-7. In the present study we found that immediate early gene cyr61 is expressed in a rapid and transient manner by bFGF during the differentiation of H19-7 cells. To clarify the

25   signal transduction pathway for the induction of cyr61 by bFGF, we checked whether Raf-1 and mitogen-activated protein kinase (MAPK) is activated during the induction of cyr61. It is identified that cyr61 is induced by bFGF via at least two signaling pathways; MAPK-dependent as well as MAPK-independent signaling pathways. This study suggested that cyr61 is likely to play an important role in neuronal differentiation process.

30   **Panel 2D Summary:** Ag1148 The expression of the CG55688-01 gene was assessed in two independent runs in panel 2D with excellent concordance between runs. The highest expression of this gene is found in normal bladder tissue and a kidney cancer sample (CTs=28). In addition, there appears to be substantial expression associated with ovarian derived tissue, prostate derived tissue and a number of kidney samples. Thus, the expression of

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this gene could be used to distinguish the bladder and kidney samples from the rest of the samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, protein therapeutics or antibodies might be beneficial in the treatment of kidney cancer, ovarian cancer or prostate cancer.

Panel 3D Summary: Ag1148 The expression of the CG55688-01 gene appears to be highest in a sample derived from a lung cancer (NCI-H292)(CT=28). In addition, there is substantial expression associated with a number of brain cancers, renal cancer cell lines, pancreatic cancer cell lines, bladder cancer cell lines and two sarcoma cell lines. Thus, the expression of this gene could be used to distinguish NCI-H292 cells from the other samples in the panel.

Panel 4D Summary: Ag1148 The CG55688-01 gene, a Cyr61 homolog, is expressed at moderate levels (CT range 28-32), in resting and cytokine-stimulated HUVEC, lung · microvascular endothelial cells, coronary artery smooth muscle cells, bronchial epithelial cells, small airway epithelial cells, astrocytes, pulmonary artery endothelial cells, lung fibroblasts, and dermal fibroblasts. Based on the expression pattern above and our understanding of the functions of Cyr61 in vascular biology (see reference), it can be concluded that antibodies and small molecule antagonists that block the function of the CG55688-01 gene product may reduce or eliminate the symptoms in patients with any of several inflammatory or autoimmune diseases, including Crohn's disease, ulcerative colitis, multiple sclerosis, chronic obstructive pulmonary disease, asthma, emphysema, rheumatoid arthritis, or psoriasis.

Reference:

Babic AM, Kireeva ML, Kolesnikova TV, Lau LF CYR61, a product of a growth factor-inducible immediate early gene, promotes angiogenesis and tumor growth. Proc Natl Acad Sci U S A 1998 May 26;95(11):6355-60

CYR61 is a secreted, cysteine-rich, heparin-binding protein encoded by a growth factor-inducible immediate-early gene. Acting as an extracellular, matrix-associated signaling molecule, CYR61 promotes the adhesion of endothelial cells through interaction with the integrin alphaVbeta3 and augments growth factor-induced DNA synthesis in the same cell type. In this study, we show that purified CYR61 stimulates directed migration of human microvascular endothelial cells in culture through an alphaV beta3-dependent pathway and induces neovascularization in rat corneas. Both the chemotactic and angiogenic activities of CYR61 can be blocked by specific anti-CYR61 antibodies. Whereas most human tumor-derived cell lines tested express CYR61, the gastric adenocarcinoma cell line RF-1 does not.

Expression of the CYR61 cDNA under the regulation of a constitutive promoter in RF-1 cells significantly enhances the tumorigenicity of these cells as measured by growth in immunodeficient mice, resulting in tumors that are larger and more vascularized than those produced by control RF-1 cells. Taken together, these results identify CYR61 as an angiogenic

5   inducer that can promote tumor growth and vascularization; the results also suggest potential roles for CYR61 in physiologic and pathologic neovascularization.

Panel 5 Islet Summary: Ag1148 The CG55688-01 gene has low expression in islet tissue. It is also expressed at low levels in mesenchymal stem cells, which can be differentiated in vitro into adipocytes, chondrocytes and osteocytes. Therefore, this gene may

10   be a monoclonal antibody target for the treatment of diseases involving adipose, cartilage or bone.

SEC6 (CG56157-01)

Expression of gene CG56157-01 was assessed using the primer-probe set Ag1102, described in Table 24A. Results of the RTQ-PCR runs are shown in Table 24B.

15   Table 24A. Probe Name Ag1102

Primers	Sequences	Length	Start Position
Forward	5'-aggatccaggaaacgaagtg-3' (SEQ ID NO:156)	20	123
Probe	TET-5'-tctacgcgctatataagcaggccactg-3'-TAMRA (SEQ ID NO:157)	27	153
Reverse	5'-gggcatgttacaaggtcctt-3' (SEQ ID NO:158)	20	180

Table 24B. Panel 1.2

Tissue Name	Rel. Exp.(%) Ag1102, Run 125939695	Tissue Name	Rel. Exp.(%) Ag1102, Run 125939695
Endothelial cells	12.3	Renal ca. 786-0	3.4
Heart (Fetal)	6.3	Renal ca. A498	5.4
Pancreas	16.4	Renal ca. RXF 393	3.2
Pancreatic ca. CAPAN 2	0.4	Renal ca. ACHN	4.8
Adrenal Gland	53.6	Renal ca. UO-31	2.2
Thyroid	12.3	Renal ca. TK-10	4.6
Salivary gland	16.3	Liver	29.9
Pituitary gland	13.4	Liver (fetal)	11.9
Brain (fetal)	4.4	Liver ca. (hepatoblast) HepG2	16.6
Brain (whole)	7.9	Lung	4.0
Brain (amygdala)	4.2	Lung (fetal)	1.7
Brain (cerebellum)	7.5	Lung ca. (small cell) LX-1	6.0
Brain (hippocampus)	14.6	Lung ca. (small cell) NCI-H69	2.0
Brain (thalamus)	4.4	Lung ca. (s.cell var.) SHP-77	3.0
Cerebral Cortex	7.0	Lung ca. (large cell)NCI-H460	10.6

Spinal cord	2.7	Lung ca. (non-sm. cell) A549	9.6
glio/astro U87-MG	3.6	Lung ca. (non-s.cell) NCI-H23	4.1
glio/astro U-118-MG	6.4	Lung ca. (non-s.cell) HOP-62	11.6
astrocytoma SW1783	4.0	Lung ca. (non-s.cl) NCI-H522	38.7
neuro*; met SK-N-AS	6.9	Lung ca. (squam.) SW 900	12.2
astrocytoma SF-539	3.7	Lung ca. (squam.) NCI-H596	3.0
astrocytoma SNB-75	1.1	Mammary gland	5.6
glioma SNB-19	3.0	Breast ca.* (pl.ef) MCF-7	3.3
glioma U251	1.7	Breast ca.* (pl.ef) MDA-MB-231	6.2
glioma SF-295	4.6	Breast ca.* (pl. ef) T47D	9.6
Heart	32.5	Breast ca. BT-549	12.2
Skeletal Muscle	**100.0**	Breast ca. MDA-N	1.5
Bone marrow	2.9	Ovary	4.4
Thymus	1.1	Ovarian ca. OVCAR-3	1.4
Spleen	1.3	Ovarian ca. OVCAR-4	15.6
Lymph node	2.0	Ovarian ca. OVCAR-5	4.7
Colorectal Tissue	2.1	Ovarian ca. OVCAR-8	9.5
Stomach	14.5	Ovarian ca. IGROV-1	5.4
Small intestine	6.9	Ovarian ca. (ascites) SK-OV-3	9.4
Colon ca. SW480	1.7	Uterus	6.4
Colon ca.* SW620 (SW480 met)	5.0	Placenta	10.7
Colon ca. HT29	0.3	Prostate	14.5
Colon ca. HCT-116	6.0	Prostate ca.* (bone met) PC-3	57.0
Colon ca. CaCo-2	5.8	Testis	14.1
Colon ca. Tissue (ODO3866)	0.6	Melanoma Hs688(A).T	3.3
Colon ca. HCC-2998	2.7	Melanoma* (met) Hs688(B).T	3.4
Gastric ca.* (liver met) NCI-N87	3.2	Melanoma UACC-62	13.8
Bladder	16.7	Melanoma M14	9.9
Trachea	1.8	Melanoma LOX IMVI	24.8
Kidney	43.2	Melanoma* (met) SK-MEL-5	19.5
Kidney (fetal)	8.1		

Panel 1.2 Summary: Ag1102 Highest expression of the CG56157-01 gene is seen in skeletal muscle (CT=24), with high levels of expression also seen in pancreas, adrenal, pituitary, heart, and liver. Diazepam binding inhibitor (DBI) is a 10-kDa polypeptide that regulates mitochondrial steroidogenesis, glucose-induced insulin secretion, metabolism of

acyl-CoA esters, fatty acid oxidation, and the action of gamma-aminobutyrate on GABAA receptors. This gene, a DBI-related protein, may thus be a small molecule target for the treatment of metabolic and endocrine diseases, including obesity and Types 1 and 2 diabetes.

This gene also expressed at high levels in all CNS regions examined. The diazepam binding inhibitor has been implicated in seizure disorders, drug dependence and memory. In addition, this ligand acts at the GABA-A receptor which has been implicated in schizophrenia and bipolar disorder. Therefore, therapeutic modulation of this gene, a diazepam binding inhibitor homolog, may be of use in any of these clinical conditions.

References:

Kolmer M, Alho H, Costa E, Pani L. Cloning and tissue-specific functional characterization of the promoter of the rat diazepam binding inhibitor, a peptide with multiple biological actions. Proc Natl Acad Sci U S A. 1993 Sep 15;90(18):8439-43.

Diazepam binding inhibitor (DBI) is a 10-kDa polypeptide that regulates mitochondrial steroidogenesis, glucose-induced insulin secretion, metabolism of acyl-CoA esters, and the action of gamma-aminobutyrate on GABAA receptors. To investigate the regulation of DBI gene expression, three positive clones were isolated from a rat genomic library. One of them contained a DBI genomic DNA fragment encompassing 4 kb of the 5' untranslated region, the first two exons, and part of the second intron of the DBI gene. Two other overlapping clones contained a processed DBI pseudogene. Several transcription initiation sites were detected by RNase protection and primer extension assays. Different tissues exhibited clear differences in the efficiencies of transcription startpoint usage. Transient expression experiments using DNA fragments of different length from the 5' untranslated region of the DBI gene showed that basal promoter activity required 146 bp of the proximal DBI sequence, whereas full activation was achieved with 423 bp of the 5' untranslated region. DNase I protection experiments with liver nuclear proteins demonstrated three protected regions at nt -387 to -333, -295 to -271, and -176 to -139 relative to the ATG initiation codon; in other tissues the pattern of protection was different. In gel shift assays the most proximal region (-176 to -139) was found to bind several general transcription factors as well as cell type-restricted nuclear proteins which may be related to specific regulatory patterns in different tissues. Thus, the DBI gene possesses some features of a housekeeping gene but also includes a variable regulation which appears to change with the function that it subserves in different cell types.

PMID: 7690962

Ferrarese C, Cogliati T, Tortorella R, Zucca C, Bogliun G, Beghi E, Passoni D, Zoia C, Begni B, Airoldi L, Alho H, Frattola L.Diazepam binding inhibitor (DBI) in the plasma of pediatric and adult epileptic patients. Epilepsy Res 1998 Jan;29(2):129-34

The polypeptide diazepam binding inhibitor (DBI) displays epileptogenic activity by
5   binding to benzodiazepine receptors. We analyzed DBI concentrations in the plasma of pediatric and adult epileptic patients, as a possible peripheral marker in epilepsy. DBI plasma concentrations are significantly higher (+ 62%, P < 0.001) in adult patients and slightly but significantly higher (+15%, P < 0.01) in pediatric patients, compared to age-related controls. Strikingly, plasma DBI is much higher (+81%, P < 0.001) in generalized epilepsy in adults and
10   in drug-resistant pediatric and adult patients. Based on these findings, plasma DBI may be considered as a peripheral biological marker of epilepsy and, in association with lymphocyte benzodiazepine receptor density, of anticonvulsant drug responsiveness.

Herzog CD, Stackman RW, Walsh TJ.Intraseptal flumazenil enhances, while diazepam binding inhibitor impairs, performance in a working memory task. Neurobiol Learn Mem
15   1996 Nov;66(3):341-52

GABAA/benzodiazepine receptors in the medial septum modulate the activity of cholinergic neurons that innervate the hippocampus. Injection of benzodiazepine (BDZ) agonists into the medial septum impairs working memory performance and decreases high-affinity choline transport (HAChT) in the hippocampus. In contrast, intraseptal injection of the
20   BDZ antagonist flumazenil increases HAChT and prevents the memory deficits induced by systemic BDZs. The present studies attempted to further characterize the behavioral effects of medial septal injections of flumazenil to an endogenous negative modulator of the GABAA/BDZ receptor complex, diazepam binding inhibitor (DBI). Male Sprague-Dawley rats were cannulated to study the effects of intraseptal injections of these BDZ ligands on
25   spatial working memory, anxiety-related behaviors in the elevated plus maze, and on general locomotor activity. Intraseptal flumazenil (10 nmol/0.5 microliter) produced a delay-dependent enhancement of DNMTS performance after an 8-h, but not a 4-h, delay interval. This promnestic dose of flumazenil had no effect on locomotor activity and did not produce changes in measures of anxiety on the plus maze. Intraseptal injection of DBI had no effect (8
30   nmol/0.5 microliter) or slightly impaired (4 nmol/0.5 microliter) DNMTS radial maze performance following an 8-h delay, without producing changes in locomotion or plus maze behavior. These data demonstrate that flumazenil has a unique profile of activity in enhancing working memory following intraseptal injection.

Ohkuma S, Katsura M, Tsujimura A. Alterations in cerebral diazepam binding inhibitor expression in drug dependence: a possible biochemical alteration common to drug dependence. Life Sci 2001 Feb 2;68(11):1215-22

5   Mechanisms for formation of drug dependence and expression of withdrawal syndrome have not fully clarified despite of huge accumulation of experimental and clinical data at present. Several clinical features of withdrawal syndrome are considered to be common among patients with drug dependence induced by different drugs of abuse. One of them is anxiety. Recent investigations have revealed that diazepam binding inhibitor (DBI), a peptide consisting of 87 amino acids with molecular weight of about 10 kDa, serves as an inverse

10   agonist for benzodiazepine (BZD) receptors with endogenously anxiogenic potential. These lines of data suggest that cerebral DBI expression in brain may participates in formation of drug dependence and/or emergence of withdrawal syndrome. Based on this working hypothesis, we have examined DBI expression in the brain derived from mice depended on alcohol (ethanol), nicotine, and morphine to investigate functional relationship between

15   cerebral DBI expression and drug dependence. Cerebral DBI expression significantly increases in animals with drug dependence induced by these drugs, and in the cases of nicotine- and morphine-dependent mice concomitant administration of antagonists for nicotinic acetylcholine and opioid receptors, respectively, abolished the increase. Abrupt cessation of administration of drugs facilitated further increase in DBI expression. Therefore,

20   these alterations in DBI expression have close relationship with formation of drug dependence and/or emergence of withdrawal syndrome, and are considered to be a common biochemical process in drug dependence induced by different drugs of abuse. Finding and elucidation of mechanisms for common biochemical alterations among drug dependence may provide a clue to clarify mechanisms for formation of drug dependence and/or emergence of withdrawal

25   syndrome.

SEC2 (CG54933-01)

Expression of gene CG54933-01 was assessed using the primer-probe set Ag2044, described in Table 25A. Results of the RTQ-PCR runs are shown in Tables 25B, 25C, 25D, 25E and 25F.

30   Table 25A. Probe Name Ag2044

Primers	Sequences	Length	Start Position
Forward	5'-gcagctggacgtcctctatc-3' (SEQ ID NO:159)	20	1530
Probe	TET-5'-ccagaacatgaacgggtccgaatact-3'-TAMRA (SEQ ID NO:160)	26	1569
Reverse	5'-ccaggaaggactggatcttc-3' (SEQ ID NO:161)	20	1599

Table 25B. General_screening_panel_v1.4

Tissue Name	Rel. Exp.(%) Ag2044, Run 208014892	Tissue Name	Rel. Exp.(%) Ag2044, Run 208014892
Adipose	2.7	Renal ca. TK-10	0.0
Melanoma* Hs688(A).T	0.0	Bladder	4.5
Melanoma* Hs688(B).T	0.0	Gastric ca. (liver met.) NCI-N87	**100.0**
Melanoma* M14	0.0	Gastric ca. KATO III	17.2
Melanoma* LOXIMVI	0.0	Colon ca. SW-948	13.0
Melanoma* SK-MEL-5	0.0	Colon ca. SW480	2.3
Squamous cell carcinoma SCC-4	2.9	Colon ca.* (SW480 met) SW620	3.7
Testis Pool	0.2	Colon ca. HT29	0.4
Prostate ca.* (bone met) PC-3	0.3	Colon ca. HCT-116	5.0
Prostate Pool	0.0	Colon ca. CaCo-2	0.2
Placenta	0.5	Colon cancer tissue	7.2
Uterus Pool	0.4	Colon ca. SW1116	5.8
Ovarian ca. OVCAR-3	25.3	Colon ca. Colo-205	1.4
Ovarian ca. SK-OV-3	3.6	Colon ca. SW-48	2.0
Ovarian ca. OVCAR-4	0.7	Colon Pool	1.5
Ovarian ca. OVCAR-5	3.9	Small Intestine Pool	0.6
Ovarian ca. IGROV-1	0.2	Stomach Pool	0.1
Ovarian ca. OVCAR-8	64.6	Bone Marrow Pool	0.4
Ovary	1.9	Fetal Heart	0.2
Breast ca. MCF-7	0.0	Heart Pool	0.3
Breast ca. MDA-MB-231	2.5	Lymph Node Pool	1.2
Breast ca. BT 549	0.2	Fetal Skeletal Muscle	0.0
Breast ca. T47D	5.5	Skeletal Muscle Pool	0.0
Breast ca. MDA-N	0.0	Spleen Pool	0.0
Breast Pool	0.8	Thymus Pool	0.0
Trachea	3.3	CNS cancer (glio/astro) U87-MG	0.0
Lung	0.1	CNS cancer (glio/astro) U-118-MG	0.0
Fetal Lung	10.7	CNS cancer (neuro;met) SK-N-AS	0.0
Lung ca. NCI-N417	0.0	CNS cancer (astro) SF-539	0.0
Lung ca. LX-1	23.2	CNS cancer (astro) SNB-75	0.1
Lung ca. NCI-H146	0.0	CNS cancer (glio) SNB-19	0.1
Lung ca. SHP-77	0.0	CNS cancer (glio) SF-295	0.0
Lung ca. A549	0.2	Brain (Amygdala) Pool	0.1
Lung ca. NCI-H526	0.0	Brain (cerebellum)	0.1
Lung ca. NCI-H23	0.0	Brain (fetal)	0.0
Lung ca. NCI-H460	0.0	Brain (Hippocampus) Pool	0.2
Lung ca. HOP-62	0.2	Cerebral Cortex Pool	0.1
Lung ca. NCI-H522	0.1	Brain (Substantia nigra) Pool	0.1
Liver	0.0	Brain (Thalamus) Pool	0.0
Fetal Liver	0.1	Brain (whole)	0.0
Liver ca. HepG2	1.1	Spinal Cord Pool	0.2

Kidney Pool	0.7	Adrenal Gland	0.0
Fetal Kidney	0.6	Pituitary gland Pool	0.0
Renal ca. 786-0	0.0	Salivary Gland	0.1
Renal ca. A498	0.1	Thyroid (female)	0.0
Renal ca. ACHN	0.0	Pancreatic ca. CAPAN2	67.4
Renal ca. UO-31	0.4	Pancreas Pool	1.3

Table 25C. Panel 1.3D

Tissue Name	Rel. Exp.(%) Ag2044, Run 150924718	Rel. Exp.(%) Ag2044, Run 151268419	Tissue Name	Rel. Exp.(%) Ag2044, Run 150924718	Rel. Exp.(%) Ag2044, Run 151268419
Liver adenocarcinoma	72.2	50.3	Kidney (fetal)	4.8	2.2
Pancreas	0.1	0.5	Renal ca. 786-0	0.0	0.0
Pancreatic ca. CAPAN 2	38.7	43.5	Renal ca. A498	0.2	0.1
Adrenal gland	0.0	0.1	Renal ca. RXF 393	0.0	0.0
Thyroid	0.0	0.0	Renal ca. ACHN	0.1	0.4
Salivary gland	0.8	0.2	Renal ca. UO-31	0.2	0.1
Pituitary gland	0.2	0.4	Renal ca. TK-10	0.0	0.0
Brain (fetal)	0.0	0.0	Liver	0.0	0.0
Brain (whole)	0.2	0.5	Liver (fetal)	0.4	0.0
Brain (amygdala)	0.9	0.5	Liver ca. (hepatoblast) HepG2	1.1	1.1
Brain (cerebellum)	0.0	0.0	Lung	28.9	17.1
Brain (hippocampus)	1.6	1.6	Lung (fetal)	57.4	40.9
Brain (substantia nigra)	1.5	0.4	Lung ca. (small cell) LX-1	20.6	17.6
Brain (thalamus)	0.6	0.6	Lung ca. (small cell) NCI-H69	0.0	0.0
Cerebral Cortex	0.2	0.1	Lung ca. (s.cell var.) SHP-77	0.0	0.0
Spinal cord	0.3	0.4	Lung ca. (large cell)NCI-H460	0.0	0.0
glio/astro U87-MG	0.0	0.0	Lung ca. (non-sm. cell) A549	0.0	0.1
glio/astro U-118-MG	0.0	0.0	Lung ca. (non-s.cell) NCI-H23	0.2	0.0
astrocytoma SW1783	0.0	0.0	Lung ca. (non-s.cell) HOP-62	0.7	0.3
neuro*; met SK-N-AS	0.0	0.0	Lung ca. (non-s.cl) NCI-H522	0.9	0.5
astrocytoma SF-539	0.0	0.1	Lung ca. (squam.) SW 900	0.0	0.0
astrocytoma SNB-75	0.0	0.0	Lung ca. (squam.) NCI-H596	0.0	0.0
glioma SNB-19	0.4	0.0	Mammary gland	0.3	0.4
glioma U251	0.0	0.0	Breast ca.* (pl.ef) MCF-7	0.0	0.0
glioma SF-295	0.4	0.1	Breast ca.*	6.4	3.1

			(pl.ef) MDA-MB-231		
Heart (fetal)	2.7	3.5	Breast ca.* (pl.ef) T47D	0.0	0.1
Heart	1.9	0.2	Breast ca. BT-549	4.2	3.6
Skeletal muscle (fetal)	0.2	0.2	Breast ca. MDA-N	0.0	0.0
Skeletal muscle	0.0	0.0	Ovary	12.2	10.2
Bone marrow	0.0	0.0	Ovarian ca. OVCAR-3	9.3	8.8
Thymus	0.2	0.2	Ovarian ca. OVCAR-4	0.8	0.4
Spleen	1.5	0.4	Ovarian ca. OVCAR-5	2.1	2.8
Lymph node	0.0	0.4	Ovarian ca. OVCAR-8	37.6	26.6
Colorectal	1.8	1.9	Ovarian ca. IGROV-1	0.0	0.0
Stomach	3.0	1.9	Ovarian ca.* (ascites) SK-OV-3	2.0	1.3
Small intestine	4.7	5.1	Uterus	2.9	1.4
Colon ca. SW480	6.0	3.2	Placenta	7.6	5.5
Colon ca.* SW620(SW480 met)	2.0	2.2	Prostate	0.8	0.8
Colon ca. HT29	0.3	0.3	Prostate ca.* (bone met)PC-3	0.2	0.3
Colon ca. HCT-116	1.8	0.9	Testis	0.7	1.7
Colon ca. CaCo-2	0.2	0.3	Melanoma Hs688(A).T	0.0	0.1
Colon ca. tissue(ODO3866)	8.0	6.8	Melanoma* (met) Hs688(B).T	0.0	0.0
Colon ca. HCC-2998	18.7	15.0	Melanoma UACC-62	0.0	0.0
Gastric ca.* (liver met) NCI-N87	**100.0**	**100.0**	Melanoma M14	0.0	0.0
Bladder	4.7	2.4	Melanoma LOX IMVI	0.0	0.0
Trachea	48.6	31.9	Melanoma* (met) SK-MEL-5	0.0	0.0
Kidney	0.4	0.4	Adipose	18.0	18.0

Table 25D. Panel 2D

Tissue Name	Rel. Exp.(%) Ag2044, Run 150925245	Rel. Exp.(%) Ag2044, Run 151268239	Tissue Name	Rel. Exp.(%) Ag2044, Run 150925245	Rel. Exp.(%) Ag2044, Run 151268239
Normal Colon	0.1	0.2	Kidney Margin 8120608	0.3	0.1
CC Well to Mod Diff (ODO3866)	3.4	3.3	Kidney Cancer 8120613	0.0	0.0
CC Margin (ODO3866)	0.1	0.3	Kidney Margin 8120614	0.6	0.8

CC Gr.2 rectosigmoid (ODO3868)	0.3	0.5	Kidney Cancer 9010320	3.8	5.0
CC Margin (ODO3868)	0.0	0.0	Kidney Margin 9010321	0.0	0.2
CC Mod Diff (ODO3920)	0.4	0.2	Normal Uterus	0.3	0.4
CC Margin (ODO3920)	0.3	0.7	Uterus Cancer 064011	1.7	2.7
CC Gr.2 ascend colon (ODO3921)	3.1	3.7	Normal Thyroid	0.0	0.0
CC Margin (ODO3921)	0.4	0.7	Thyroid Cancer 064010	0.0	0.0
CC from Partial Hepatectomy (ODO4309) Mets	24.8	28.7	Thyroid Cancer A302152	0.0	0.0
Liver Margin (ODO4309)	0.0	0.0	Thyroid Margin A302153	0.0	0.1
Colon mets to lung (OD04451-01)	0.4	0.7	Normal Breast	0.0	0.0
Lung Margin (OD04451-02)	2.4	2.2	Breast Cancer (OD04566)	0.1	0.0
Normal Prostate 6546-1	0.3	0.7	Breast Cancer (OD04590-01)	0.0	0.0
Prostate Cancer (OD04410)	0.0	0.0	Breast Cancer Mets (OD04590-03)	0.0	0.0
Prostate Margin (OD04410)	0.0	0.2	Breast Cancer Metastasis (OD04655-05)	0.2	0.2
Prostate Cancer (OD04720-01)	2.8	3.1	Breast Cancer 064006	1.2	1.0
Prostate Margin (OD04720-02)	1.6	1.0	Breast Cancer 1024	0.0	0.1
Normal Lung 061010	6.0	6.1	Breast Cancer 9100266	0.0	0.1
Lung Met to Muscle (ODO4286)	1.4	1.1	Breast Margin 9100265	0.0	0.1
Muscle Margin (ODO4286)	0.0	0.1	Breast Cancer A209073	0.0	0.0
Lung Malignant Cancer (OD03126)	10.6	11.7	Breast Margin A2090734	0.0	0.0
Lung Margin (OD03126)	10.9	14.5	Normal Liver	0.0	0.0
Lung Cancer (OD04404)	1.7	3.1	Liver Cancer 064003	0.1	0.0
Lung Margin (OD04404)	12.7	16.4	Liver Cancer 1025	0.0	0.0
Lung Cancer (OD04565)	0.0	0.0	Liver Cancer 1026	0.0	0.0
Lung Margin (OD04565)	3.2	4.7	Liver Cancer 6004-T	0.0	0.0
Lung Cancer (OD04237-01)	0.1	0.4	Liver Tissue 6004-N	0.0	0.0
Lung Margin	2.7	3.3	Liver Cancer	0.0	0.0

(OD04237-02)			6005-T		
Ocular Mel Met to Liver (ODO4310)	0.0	0.0	Liver Tissue 6005-N	0.0	0.0
Liver Margin (ODO4310)	0.0	0.0	Normal Bladder	3.1	4.8
Melanoma Mets to Lung (OD04321)	0.1	0.0	Bladder Cancer 1023	5.6	7.7
Lung Margin (OD04321)	5.1	5.0	Bladder Cancer A302173	0.3	0.3
Normal Kidney	0.6	0.7	Bladder Cancer (OD04718-01)	0.6	0.9
Kidney Ca, Nuclear grade 2 (OD04338)	0.1	0.0	Bladder Normal Adjacent (OD04718-03)	0.0	0.0
Kidney Margin (OD04338)	0.2	0.2	Normal Ovary	2.6	3.9
Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	0.0	Ovarian Cancer 064008	20.9	24.3
Kidney Margin (OD04339)	0.2	0.2	Ovarian Cancer (OD04768-07)	**100.0**	**100.0**
Kidney Ca, Clear cell type (OD04340)	0.4	0.4	Ovary Margin (OD04768-08)	0.8	1.9
Kidney Margin (OD04340)	0.0	0.3	Normal Stomach	0.1	0.1
Kidney Ca, Nuclear grade 3 (OD04348)	0.0	0.0	Gastric Cancer 9060358	0.3	0.7
Kidney Margin (OD04348)	0.2	0.4	Stomach Margin 9060359	0.1	0.2
Kidney Cancer (OD04622-01)	8.2	12.9	Gastric Cancer 9060395	17.1	18.7
Kidney Margin (OD04622-03)	0.1	0.3	Stomach Margin 9060394	11.6	13.5
Kidney Cancer (OD04450-01)	0.1	0.3	Gastric Cancer 9060397	24.3	29.3
Kidney Margin (OD04450-03)	0.2	0.2	Stomach Margin 9060396	0.8	1.3
Kidney Cancer 8120607	0.0	0.0	Gastric Cancer 064005	0.6	0.9

Table 25E. Panel 3D

Tissue Name	Rel. Exp.(%) Ag2044, Run 170745401	Tissue Name	Rel. Exp.(%) Ag2044, Run 170745401
Daoy- Medulloblastoma	0.0	Ca Ski- Cervical epidermoid carcinoma (metastasis)	17.2
TE671- Medulloblastoma	0.0	ES-2- Ovarian clear cell carcinoma	0.0
D283 Med- Medulloblastoma	0.0	Ramos- Stimulated with PMA/ionomycin 6h	0.1
PFSK-1- Primitive Neuroectodermal	0.0	Ramos- Stimulated with PMA/ionomycin 14h	0.0
XF-498- CNS	0.0	MEG-01- Chronic myelogenous leukemia (megokaryoblast)	0.0
SNB-78- Glioma	0.0	Raji- Burkitt's lymphoma	0.0

SF-268- Glioblastoma	0.1	Daudi- Burkitt's lymphoma	0.0
T98G- Glioblastoma	0.0	U266- B-cell plasmacytoma	0.0
SK-N-SH- Neuroblastoma (metastasis)	0.0	CA46- Burkitt's lymphoma	0.0
SF-295- Glioblastoma	0.0	RL- non-Hodgkin's B-cell lymphoma	0.0
Cerebellum	0.0	JM1- pre-B-cell lymphoma	0.5
Cerebellum	0.0	Jurkat- T cell leukemia	0.0
NCI-H292- Mucoepidermoid lung carcinoma	44.4	TF-1- Erythroleukemia	0.0
DMS-114- Small cell lung cancer	0.0	HUT 78- T-cell lymphoma	0.0
DMS-79- Small cell lung cancer	**100.0**	U937- Histiocytic lymphoma	0.0
NCI-H146- Small cell lung cancer	0.0	KU-812- Myelogenous leukemia	0.0
NCI-H526- Small cell lung cancer	0.0	769-P- Clear cell renal carcinoma	0.0
NCI-N417- Small cell lung cancer	0.0	Caki-2- Clear cell renal carcinoma	0.0
NCI-H82- Small cell lung cancer	0.0	SW 839- Clear cell renal carcinoma	0.0
NCI-H157- Squamous cell lung cancer (metastasis)	0.0	G401- Wilms' tumor	0.1
NCI-H1155- Large cell lung cancer	0.0	Hs766T- Pancreatic carcinoma (LN metastasis)	14.1
NCI-H1299- Large cell lung cancer	0.0	CAPAN-1- Pancreatic adenocarcinoma (liver metastasis)	2.0
NCI-H727- Lung carcinoid	0.0	SU86.86- Pancreatic carcinoma (liver metastasis)	0.5
NCI-UMC-11- Lung carcinoid	0.0	BxPC-3- Pancreatic adenocarcinoma	13.6
LX-1- Small cell lung cancer	40.6	HPAC- Pancreatic adenocarcinoma	34.6
Colo-205- Colon cancer	7.4	MIA PaCa-2- Pancreatic carcinoma	0.1
KM12- Colon cancer	0.0	CFPAC-1- Pancreatic ductal adenocarcinoma	19.9
KM20L2- Colon cancer	11.7	PANC-1- Pancreatic epithelioid ductal carcinoma	0.6
NCI-H716- Colon cancer	0.0	T24- Bladder carcinma (transitional cell)	0.1
SW-48- Colon adenocarcinoma	4.5	5637- Bladder carcinoma	0.3
SW1116- Colon adenocarcinoma	17.6	HT-1197- Bladder carcinoma	0.1
LS 174T- Colon adenocarcinoma	72.7	UM-UC-3- Bladder carcinma (transitional cell)	0.0
SW-948- Colon adenocarcinoma	3.1	A204- Rhabdomyosarcoma	0.0
SW-480- Colon adenocarcinoma	0.4	HT-1080- Fibrosarcoma	0.0
NCI-SNU-5- Gastric carcinoma	5.1	MG-63- Osteosarcoma	0.1
KATO III- Gastric carcinoma	0.0	SK-LMS-1- Leiomyosarcoma (vulva)	0.0

NCI-SNU-16- Gastric carcinoma	0.0	SJRH30- Rhabdomyosarcoma (met to bone marrow)	0.0
NCI-SNU-1- Gastric carcinoma	0.1	A431- Epidermoid carcinoma	0.0
RF-1- Gastric adenocarcinoma	0.0	WM266-4- Melanoma	0.0
RF-48- Gastric adenocarcinoma	0.0	DU 145- Prostate carcinoma (brain metastasis)	0.0
MKN-45- Gastric carcinoma	22.5	MDA-MB-468- Breast adenocarcinoma	0.0
NCI-N87- Gastric carcinoma	53.6	SCC-4- Squamous cell carcinoma of tongue	0.0
OVCAR-5- Ovarian carcinoma	4.8	SCC-9- Squamous cell carcinoma of tongue	0.0
RL95-2- Uterine carcinoma	8.0	SCC-15- Squamous cell carcinoma of tongue	0.0
HelaS3- Cervical adenocarcinoma	18.6	CAL 27- Squamous cell carcinoma of tongue	0.2

Table 25F. Panel 4D

Tissue Name	Rel. Exp.(%) Ag2044, Run 150925280	Rel. Exp.(%) Ag2044, Run 151536254	Tissue Name	Rel. Exp.(%) Ag2044, Run 150925280	Rel. Exp.(%) Ag2044, Run 151536254
Secondary Th1 act	0.0	0.0	HUVEC IL-1beta	0.1	0.0
Secondary Th2 act	0.0	0.0	HUVEC IFN gamma	0.0	0.0
Secondary Tr1 act	0.0	0.0	HUVEC TNF alpha + IFN gamma	0.0	0.0
Secondary Th1 rest	0.2	0.0	HUVEC TNF alpha + IL4	0.0	0.0
Secondary Th2 rest	0.0	0.0	HUVEC IL-11	0.0	0.0
Secondary Tr1 rest	0.2	0.0	Lung Microvascular EC none	0.0	0.0
Primary Th1 act	0.0	0.0	Lung Microvascular EC TNFalpha + IL-1beta	0.0	0.0
Primary Th2 act	0.0	0.0	Microvascular Dermal EC none	0.0	0.0
Primary Tr1 act	0.0	0.0	Microsvasular Dermal EC TNFalpha + IL-1beta	0.0	0.0
Primary Th1 rest	0.0	0.0	Bronchial epithelium TNFalpha + IL1beta	0.6	6.3
Primary Th2 rest	0.0	0.0	Small airway epithelium none	0.3	1.6
Primary Tr1 rest	0.3	0.0	Small airway epithelium TNFalpha + IL-1beta	0.6	2.3
CD45RA CD4 lymphocyte act	0.0	0.0	Coronery artery SMC rest	0.0	0.7
CD45RO CD4 lymphocyte act	0.0	0.0	Coronery artery SMC TNFalpha + IL-1beta	0.0	0.0
CD8 lymphocyte act	0.0	0.0	Astrocytes rest	0.2	0.4
Secondary CD8 lymphocyte rest	0.0	0.0	Astrocytes TNFalpha + IL-1beta	**100.0**	3.2

Secondary CD8 lymphocyte act	0.0	0.0	KU-812 (Basophil) rest	0.0	0.0
CD4 lymphocyte none	0.2	0.0	KU-812 (Basophil) PMA/ionomycin	0.0	0.0
2ry Th1/Th2/Tr1_anti-CD95 CH11	0.0	0.0	CCD1106 (Keratinocytes) none	0.1	1.2
LAK cells rest	0.0	0.0	CCD1106 (Keratinocytes) TNFalpha + IL-1beta	0.0	0.7
LAK cells IL-2	0.0	0.0	Liver cirrhosis	1.4	5.8
LAK cells IL-2+IL-12	0.0	0.0	Lupus kidney	0.0	0.4
LAK cells IL-2+IFN gamma	0.0	0.0	NCI-H292 none	24.7	75.3
LAK cells IL-2+ IL-18	0.0	0.0	NCI-H292 IL-4	32.5	95.3
LAK cells PMA/ionomycin	0.1	0.0	NCI-H292 IL-9	24.8	85.3
NK Cells IL-2 rest	0.0	0.0	NCI-H292 IL-13	24.1	**100.0**
Two Way MLR 3 day	0.0	0.0	NCI-H292 IFN gamma	22.5	75.8
Two Way MLR 5 day	0.0	0.0	HPAEC none	0.0	0.0
Two Way MLR 7 day	0.0	0.0	HPAEC TNF alpha + IL-1 beta	0.0	0.0
PBMC rest	0.0	0.0	Lung fibroblast none	0.0	0.0
PBMC PWM	0.0	0.0	Lung fibroblast TNF alpha + IL-1 beta	0.0	0.0
PBMC PHA-L	0.0	0.0	Lung fibroblast IL-4	0.0	0.0
Ramos (B cell) none	0.0	0.0	Lung fibroblast IL-9	0.0	0.0
Ramos (B cell) ionomycin	0.0	0.0	Lung fibroblast IL-13	0.0	0.0
B lymphocytes PWM	0.0	0.0	Lung fibroblast IFN gamma	0.0	0.0
B lymphocytes CD40L and IL-4	0.0	0.0	Dermal fibroblast CCD1070 rest	0.0	0.4
EOL-1 dbcAMP	0.0	0.0	Dermal fibroblast CCD1070 TNF alpha	0.0	0.0
EOL-1 dbcAMP PMA/ionomycin	0.0	0.0	Dermal fibroblast CCD1070 IL-1 beta	0.0	0.0
Dendritic cells none	0.0	0.0	Dermal fibroblast IFN gamma	0.0	0.0
Dendritic cells LPS	0.0	0.0	Dermal fibroblast IL-4	0.0	0.0
Dendritic cells anti-CD40	0.0	0.0	IBD Colitis 2	0.0	0.0
Monocytes rest	0.0	0.0	IBD Crohn's	0.0	0.0
Monocytes LPS	0.1	0.4	Colon	4.5	11.0
Macrophages rest	0.0	0.0	Lung	23.3	84.7
Macrophages LPS	0.0	0.0	Thymus	0.7	2.6
HUVEC none	0.0	0.0	Kidney	0.2	1.3
HUVEC starved	0.0	0.0			

General_screening_panel_v1.4 Summary: Ag2044 The expression of the CG54933-01 gene appears to be highest in a sample derived from a gastric cancer cell line (NCI-N87)(CT=25.1). In addition, there is substantial expression in ovarian cancer cell lines, a pancreatic cancer cell line and colon cancer cell lines. Thus, the expression of this gene could be used to distinguish NCI-N87 cells from the rest of the samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, monoclonal antibodies or protein therapeutics might be beneficial for the treatment of colon cancer, gastric cancer, pancreatic cancer or ovarian cancer.

The expression of the isoform of this gene in endocrine/metabolically-related tissues is restricted to adipose, pancreas and small intestine. Due to its presumed role in cell adhesion and the moderate level of expression in adipose, this gene and/or gene product may be a target in the treatment of obesity.

Panel 1.3D Summary: Ag2044 The expression of the CG54933-01 gene was assessed in two independent runs on panel 1.3D with excellent concordance between the runs. The expression of this gene is highest in a sample derived from a gastric cancer cell line (NCI-N87)(CTs=28), consistent with the expression in General_screening_panel_v1.4. In addition, there is substantial expression observed in colon cancer cell lines, ovarian cancer cell lines, normal lung tissue, a liver cancer sample and a sample derived from a pancreatic cancer cell line. Thus, the expression of this gene could be used to distinguish NCI-N87 cells from other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be beneficial for the treatment of colon cancer, gastric cancer, pancreatic cancer, liver cancer or ovarian cancer.

Panel 2D Summary: Ag2044 The expression of the CG54933-01 gene was assessed in two independent runs on panel 2D with excellent concordance between the runs. Overall, the expression of this gene is highest in samples derived from ovarian cancer tissue (CTs=25). This gene encodes a protein that is homologous to mesothelin, which has been shown to be up-regulated in ovarian cancer. In addition, there is substantial expression observed in metastatic colon cancer derived tissue, lung derived tissue and gastric cancer derived tissue. Thus, the expression of this gene could be used to distinguish the above mentioned tissues from other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be beneficial for the treatment of colon cancer, gastric cancer, lung cancer or ovarian cancer.

References:

Hassan R, Viner JL, Wang QC, Margulies I, Kreitman RJ, Pastan I.Anti-tumor activity of K1-LysPE38QQR, an immunotoxin targeting mesothelin, a cell-surface antigen overexpressed in ovarian cancer and malignant mesothelioma.

J Immunother 2000 Jul-Aug;23(4):473-9

5   Mesothelin, a differentiation antigen, is a 40-kD glycosylphosphatidylinositol-linked cell-surface glycoprotein, that is present on the surface of normal mesothelium and is overexpressed in many patients with epithelial ovarian cancer and malignant mesotheliomas. Monoclonal antibody K1 is a murine immunoglobulin G1 that recognizes mesothelin. LysPE38QQR is a truncated form of Pseudomonas exotoxin that lacks the cell-binding

10   domain, but retains the translocation and adenosine diphosphate-ribosylation domains. It has a single lysine residue near the amino terminus that is available for conjugation to antibodies. To prevent chemical conjugation of the antibody to lysine residues at the C-terminus of Pseudomonas exotoxin, the two lysine residues at positions 590 and 606 were mutated to glutamine, and the lysine residue at position 613 was mutated to arginine. Monoclonal

15   antibody K1 was chemically conjugated with LysPE38QQR, by modifying the antibody with sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate and coupling it with SPDP N-succinimidyl 3-(2-pyridyldithio)propionate-modified LysPE38QQR. The resulting immunotoxin K1-LysPE38QQR was highly toxic to A431-K5 cells (a human epidermoid carcinoma cell line transfected with a mesothelin expression plasmid) with a half-maximal

20   inhibitory concentration of 3-6 ng/mL. The immunotoxin had negligible activity against A431 cells, which do not express mesothelin (median inhibitory concentration > 100 ng/mL). This immunotoxin also caused complete regression of tumors in nude mice that received xenografts of mesothelin-positive human carcinomas. These results show that immunotoxins directed against mesothelin are a therapeutic option that merits further investigation for the treatment of

25   ovarian cancer and malignant mesotheliomas.

PMID: 10916757

Panel 3D Summary: Ag2044 The expression of the CG54933-01 gene appears to be highest in a sample derived from a lung cancer cell line (DMS-79)(CT=27.8). In addition, there appears to be substantial expression in colon cancer derived cell lines, pancreatic cancer

30   derived cell lines, gastric cancer derived cell lines, lung cancer derived cell lines and cervical cancer derived cell lines. Thus, the expression of this gene could be used to distinguish DMS-79 cells from other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be beneficial

for the treatment of colon cancer, gastric cancer, lung cancer, cervical cancer, or pancreatic cancer.

Panel 4D Summary: Ag2044 The expression of the CG54933-01 gene was assessed in two independent runs using same set of primers. Both runs show moderate expression of this transcript in the NCI H292 cell line, a human airway epithelial cell line that produces mucins. Mucus overproduction is an important feature of bronchial asthma and chronic obstructive pulmonary disease(COPD). This transcript encodes for mesothelin, a cell surface protein, that may play a role in cellular adhesion or in megakaryocyte proliferation. Thus, this gene may be involved in promoting hyperplasia or mucus production in these cell type. Therefore, modulation of the expression or activity of this gene or gene product by antibodies could beneficial for the treatment of these asthma and COPD.

SEC8 (CG56010-01)

Expression of gene CG56010-01 was assessed using the primer-probe set Ag1438, described in Table 26A. Results of the RTQ-PCR runs are shown in Tables 26B, 26C, 26D, 26E and 26F.

Table 26A. Probe Name Ag1438

Primers	Sequences	Length	Start Position
Forward	5'-gccaggcactgttcatctc-3' (SEQ ID NO:162)	19	285
Probe	TET-5'-ctcccggcaagctttctgctgaaag-3'-TAMRA (SEQ ID NO:163)	25	322
Reverse	5'-gacatcaggctccagatatgaa-3' (SEQ ID NO:164)	22	347

Table 26B. Panel 1.2

Tissue Name	Rel. Exp.(%) Ag1438, Run 138373879	Tissue Name	Rel. Exp.(%) Ag1438, Run 138373879
Endothelial cells	0.1	Renal ca. 786-0	0.0
Heart (Fetal)	1.0	Renal ca. A498	0.0
Pancreas	0.2	Renal ca. RXF 393	0.0
Pancreatic ca. CAPAN 2	0.0	Renal ca. ACHN	0.0
Adrenal Gland	3.2	Renal ca. UO-31	0.0
Thyroid	22.8	Renal ca. TK-10	0.0
Salivary gland	16.7	Liver	0.4
Pituitary gland	0.1	Liver (fetal)	0.1
Brain (fetal)	0.0	Liver ca. (hepatoblast) HepG2	0.5
Brain (whole)	0.0	Lung	0.1
Brain (amygdala)	0.0	Lung (fetal)	0.1
Brain (cerebellum)	0.0	Lung ca. (small cell) LX-1	47.6
Brain (hippocampus)	0.0	Lung ca. (small cell) NCI-H69	4.6
Brain (thalamus)	0.0	Lung ca. (s.cell var.)	7.2

		SHP-77	
Cerebral Cortex	0.0	Lung ca. (large cell)NCI-H460	0.0
Spinal cord	0.0	Lung ca. (non-sm. cell) A549	0.5
glio/astro U87-MG	0.0	Lung ca. (non-s.cell) NCI-H23	0.0
glio/astro U-118-MG	0.0	Lung ca. (non-s.cell) HOP-62	0.0
astrocytoma SW1783	0.0	Lung ca. (non-s.cl) NCI-H522	0.0
neuro*; met SK-N-AS	0.0	Lung ca. (squam.) SW 900	0.1
astrocytoma SF-539	0.0	Lung ca. (squam.) NCI-H596	2.7
astrocytoma SNB-75	0.0	Mammary gland	0.7
glioma SNB-19	0.0	Breast ca.* (pl.ef) MCF-7	1.4
glioma U251	0.0	Breast ca.* (pl.ef) MDA-MB-231	0.0
glioma SF-295	0.0	Breast ca.* (pl. ef) T47D	**100.0**
Heart	1.6	Breast ca. BT-549	0.0
Skeletal Muscle	0.4	Breast ca. MDA-N	0.0
Bone marrow	2.5	Ovary	1.1
Thymus	0.0	Ovarian ca. OVCAR-3	0.0
Spleen	0.4	Ovarian ca. OVCAR-4	0.0
Lymph node	0.0	Ovarian ca. OVCAR-5	0.1
Colorectal Tissue	22.2	Ovarian ca. OVCAR-8	0.0
Stomach	0.2	Ovarian ca. IGROV-1	0.0
Small intestine	15.9	Ovarian ca. (ascites) SK-OV-3	0.0
Colon ca. SW480	0.0	Uterus	0.3
Colon ca.* SW620 (SW480 met)	4.1	Placenta	0.0
Colon ca. HT29	1.7	Prostate	3.5
Colon ca. HCT-116	0.0	Prostate ca.* (bone met) PC-3	0.1
Colon ca. CaCo-2	4.6	Testis	0.0
Colon ca. Tissue (ODO3866)	2.0	Melanoma Hs688(A).T	0.0
Colon ca. HCC-2998	0.0	Melanoma* (met) Hs688(B).T	0.0
Gastric ca.* (liver met) NCI-N87	0.0	Melanoma UACC-62	0.0
Bladder	3.6	Melanoma M14	0.0
Trachea	4.9	Melanoma LOX IMVI	0.0
Kidney	0.7	Melanoma* (met) SK-MEL-5	0.0
Kidney (fetal)	1.0		

Table 26C. Panel 1.3D

Tissue Name	Rel. Exp.(%) Ag1438, Run 146127639	Rel. Exp.(%) Ag1438, Run 151268950	Tissue Name	Rel. Exp.(%) Ag1438, Run 146127639	Rel. Exp.(%) Ag1438, Run 151268950

Liver adenocarcinoma	0.0	0.0	Kidney (fetal)	0.6	1.7
Pancreas	1.0	1.6	Renal ca. 786-0	0.1	0.0
Pancreatic ca. CAPAN 2	0.0	0.0	Renal ca. A498	0.0	0.0
Adrenal gland	1.6	1.5	Renal ca. RXF 393	0.1	0.0
Thyroid	50.3	62.4	Renal ca. ACHN	0.0	0.0
Salivary gland	4.8	9.7	Renal ca. UO-31	0.0	0.0
Pituitary gland	0.2	0.2	Renal ca. TK-10	0.0	0.0
Brain (fetal)	0.0	0.0	Liver	0.4	0.6
Brain (whole)	0.0	0.0	Liver (fetal)	0.3	0.3
Brain (amygdala)	0.0	0.1	Liver ca. (hepatoblast) HepG2	0.3	0.3
Brain (cerebellum)	0.1	0.1	Lung	3.1	4.2
Brain (hippocampus)	0.1	0.4	Lung (fetal)	3.4	4.1
Brain (substantia nigra)	0.4	0.3	Lung ca. (small cell) LX-1	39.0	33.7
Brain (thalamus)	0.1	0.1	Lung ca. (small cell) NCI-H69	11.6	14.6
Cerebral Cortex	0.1	0.1	Lung ca. (s.cell var.) SHP-77	32.1	30.8
Spinal cord	0.3	0.4	Lung ca. (large cell)NCI-H460	0.0	0.0
glio/astro U87-MG	0.0	0.0	Lung ca. (non-sm. cell) A549	0.5	0.8
glio/astro U-118-MG	0.0	0.0	Lung ca. (non-s.cell) NCI-H23	0.0	0.0
astrocytoma SW1783	0.0	0.0	Lung ca. (non-s.cell) HOP-62	0.0	0.1
neuro*; met SK-N-AS	0.1	0.2	Lung ca. (non-s.cl) NCI-H522	0.1	0.0
astrocytoma SF-539	0.0	0.0	Lung ca. (squam.) SW 900	0.1	0.0
astrocytoma SNB-75	0.2	0.1	Lung ca. (squam.) NCI-H596	4.3	3.3
glioma SNB-19	0.0	0.1	Mammary gland	13.3	11.6
glioma U251	0.0	0.0	Breast ca.* (pl.ef) MCF-7	2.7	2.2
glioma SF-295	0.0	0.0	Breast ca.* (pl.ef) MDA-MB-231	0.7	0.5
Heart (fetal)	3.0	4.7	Breast ca.* (pl.ef) T47D	**100.0**	**100.0**
Heart	0.4	0.2	Breast ca. BT-549	0.0	0.1
Skeletal muscle (fetal)	1.0	2.1	Breast ca. MDA-N	0.0	0.0
Skeletal muscle	0.2	0.1	Ovary	0.6	1.1
Bone marrow	5.1	6.2	Ovarian ca. OVCAR-3	0.0	0.0

Thymus	0.2	0.2	Ovarian ca. OVCAR-4	0.0	0.0
Spleen	2.9	3.2	Ovarian ca. OVCAR-5	0.1	0.1
Lymph node	1.6	1.3	Ovarian ca. OVCAR-8	0.0	0.0
Colorectal	22.1	31.6	Ovarian ca. IGROV-1	0.0	0.0
Stomach	2.3	2.0	Ovarian ca.* (ascites) SK-OV-3	0.0	0.0
Small intestine	21.3	25.9	Uterus	1.1	0.8
Colon ca. SW480	0.0	0.1	Placenta	0.2	0.0
Colon ca.* SW620(SW480 met)	1.7	1.4	Prostate	4.2	6.1
Colon ca. HT29	1.4	2.0	Prostate ca.* (bone met)PC-3	0.0	0.3
Colon ca. HCT-116	0.0	0.0	Testis	0.2	0.2
Colon ca. CaCo-2	6.2	6.8	Melanoma Hs688(A).T	0.1	0.0
Colon ca. tissue(ODO3866)	36.1	28.7	Melanoma* (met) Hs688(B).T	0.0	0.0
Colon ca. HCC-2998	0.0	0.0	Melanoma UACC-62	0.0	0.0
Gastric ca.* (liver met) NCI-N87	0.0	0.0	Melanoma M14	0.1	0.0
Bladder	1.4	1.8	Melanoma LOX IMVI	0.0	0.2
Trachea	68.3	56.3	Melanoma* (met) SK-MEL-5	0.0	0.0
Kidney	0.2	0.1	Adipose	3.4	3.6

Table 26D. Panel 2D

Tissue Name	Rel. Exp.(%) Ag1438, Run 145165485	Rel. Exp.(%) Ag1438, Run 145375711	Tissue Name	Rel. Exp.(%) Ag1438, Run 145165485	Rel. Exp.(%) Ag1438, Run 145375711
Normal Colon	16.3	4.6	Kidney Margin 8120608	0.1	0.1
CC Well to Mod Diff (ODO3866)	6.5	1.6	Kidney Cancer 8120613	0.0	0.0
CC Margin (ODO3866)	27.0	6.5	Kidney Margin 8120614	0.1	0.0
CC Gr.2 rectosigmoid (ODO3868)	16.4	7.7	Kidney Cancer 9010320	0.1	0.0
CC Margin (ODO3868)	0.2	0.1	Kidney Margin 9010321	0.0	0.0
CC Mod Diff (ODO3920)	8.2	5.1	Normal Uterus	0.1	0.0
CC Margin (ODO3920)	6.7	3.2	Uterus Cancer 064011	11.0	17.4
CC Gr.2 ascend colon (ODO3921)	27.9	16.0	Normal Thyroid	17.6	18.3

CC Margin (ODO3921)	14.8	6.0	Thyroid Cancer 064010	0.0	0.0
CC from Partial Hepatectomy (ODO4309) Mets	5.6	3.9	Thyroid Cancer A302152	0.1	0.1
Liver Margin (ODO4309)	0.1	0.0	Thyroid Margin A302153	36.9	41.8
Colon mets to lung (OD04451-01)	7.2	5.7	Normal Breast	0.9	0.5
Lung Margin (OD04451-02)	0.4	0.1	Breast Cancer (OD04566)	1.1	1.1
Normal Prostate 6546-1	1.5	1.0	Breast Cancer (OD04590-01)	3.5	1.0
Prostate Cancer (OD04410)	3.7	1.9	Breast Cancer Mets (OD04590-03)	13.0	12.9
Prostate Margin (OD04410)	5.8	5.4	Breast Cancer Metastasis (OD04655-05)	3.1	3.0
Prostate Cancer (OD04720-01)	0.1	0.2	Breast Cancer 064006	2.9	1.9
Prostate Margin (OD04720-02)	0.6	1.0	Breast Cancer 1024	11.4	3.5
Normal Lung 061010	2.5	2.8	Breast Cancer 9100266	27.7	33.4
Lung Met to Muscle (ODO4286)	0.0	0.0	Breast Margin 9100265	3.5	7.1
Muscle Margin (ODO4286)	0.1	0.0	Breast Cancer A209073	2.9	4.2
Lung Malignant Cancer (OD03126)	**100.0**	**100.0**	Breast Margin A2090734	1.6	0.5
Lung Margin (OD03126)	0.6	0.5	Normal Liver	0.0	0.0
Lung Cancer (OD04404)	0.1	0.1	Liver Cancer 064003	0.1	0.0
Lung Margin (OD04404)	0.7	0.1	Liver Cancer 1025	0.0	0.0
Lung Cancer (OD04565)	0.0	0.0	Liver Cancer 1026	0.1	0.0
Lung Margin (OD04565)	0.2	0.2	Liver Cancer 6004-T	0.0	0.0
Lung Cancer (OD04237-01)	1.9	1.0	Liver Tissue 6004-N	0.1	0.0
Lung Margin (OD04237-02)	0.4	0.1	Liver Cancer 6005-T	0.1	0.1
Ocular Mel Met to Liver (ODO4310)	0.0	0.0	Liver Tissue 6005-N	0.0	0.0
Liver Margin (ODO4310)	0.3	0.3	Normal Bladder	0.4	0.4
Melanoma Mets to Lung (OD04321)	0.4	0.3	Bladder Cancer 1023	12.3	7.2
Lung Margin (OD04321)	0.3	0.3	Bladder Cancer A302173	0.1	0.0
Normal Kidney	0.1	0.0	Bladder Cancer (OD04718-01)	0.0	0.0

Kidney Ca, Nuclear grade 2 (OD04338)	0.0	0.0	Bladder Normal Adjacent (OD04718-03)	0.0	0.0
Kidney Margin (OD04338)	0.1	0.0	Normal Ovary	0.2	0.1
Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	0.0	Ovarian Cancer 064008	0.1	0.0
Kidney Margin (OD04339)	0.1	0.0	Ovarian Cancer (OD04768-07)	0.1	0.1
Kidney Ca, Clear cell type (OD04340)	0.1	0.1	Ovary Margin (OD04768-08)	0.2	0.1
Kidney Margin (OD04340)	0.1	0.0	Normal Stomach	0.1	0.1
Kidney Ca, Nuclear grade 3 (OD04348)	0.0	0.0	Gastric Cancer 9060358	0.2	0.1
Kidney Margin (OD04348)	0.1	0.1	Stomach Margin 9060359	0.2	0.1
Kidney Cancer (OD04622-01)	0.1	0.1	Gastric Cancer 9060395	1.3	0.5
Kidney Margin (OD04622-03)	0.1	0.1	Stomach Margin 9060394	1.6	1.0
Kidney Cancer (OD04450-01)	0.0	0.0	Gastric Cancer 9060397	15.2	9.2
Kidney Margin (OD04450-03)	0.1	0.0	Stomach Margin 9060396	1.2	0.5
Kidney Cancer 8120607	0.0	0.0	Gastric Cancer 064005	1.7	1.1

Table 26E. Panel 3D

Tissue Name	Rel. Exp.(%) Ag1438, Run 164169778	Tissue Name	Rel. Exp.(%) Ag1438, Run 164169778
Daoy- Medulloblastoma	0.1	Ca Ski- Cervical epidermoid carcinoma (metastasis)	0.0
TE671- Medulloblastoma	0.4	ES-2- Ovarian clear cell carcinoma	0.0
D283 Med- Medulloblastoma	0.0	Ramos- Stimulated with PMA/ionomycin 6h	0.0
PFSK-1- Primitive Neuroectodermal	0.4	Ramos- Stimulated with PMA/ionomycin 14h	0.0
XF-498- CNS	1.3	MEG-01- Chronic myelogenous leukemia (megokaryoblast)	0.0
SNB-78- Glioma	0.0	Raji- Burkitt's lymphoma	0.0
SF-268- Glioblastoma	0.0	Daudi- Burkitt's lymphoma	0.0
T98G- Glioblastoma	0.0	U266- B-cell plasmacytoma	0.0
SK-N-SH- Neuroblastoma (metastasis)	0.0	CA46- Burkitt's lymphoma	0.0
SF-295- Glioblastoma	0.0	RL- non-Hodgkin's B-cell lymphoma	0.0
Cerebellum	0.1	JM1- pre-B-cell lymphoma	0.6
Cerebellum	0.0	Jurkat- T cell leukemia	0.0
NCI-H292- Mucoepidermoid lung carcinoma	0.0	TF-1- Erythroleukemia	0.0
DMS-114- Small cell lung	0.0	HUT 78- T-cell lymphoma	0.0

cancer			
DMS-79- Small cell lung cancer	17.6	U937- Histiocytic lymphoma	0.0
NCI-H146- Small cell lung cancer	65.1	KU-812- Myelogenous leukemia	0.0
NCI-H526- Small cell lung cancer	79.6	769-P- Clear cell renal carcinoma	0.0
NCI-N417- Small cell lung cancer	0.0	Caki-2- Clear cell renal carcinoma	0.0
NCI-H82- Small cell lung cancer	0.1	SW 839- Clear cell renal carcinoma	0.0
NCI-H157- Squamous cell lung cancer (metastasis)	0.0	G401- Wilms' tumor	0.0
NCI-H1155- Large cell lung cancer	26.1	Hs766T- Pancreatic carcinoma (LN metastasis)	0.6
NCI-H1299- Large cell lung cancer	0.0	CAPAN-1- Pancreatic adenocarcinoma (liver metastasis)	0.8
NCI-H727- Lung carcinoid	88.3	SU86.86- Pancreatic carcinoma (liver metastasis)	0.7
NCI-UMC-11- Lung carcinoid	**100.0**	BxPC-3- Pancreatic adenocarcinoma	0.2
LX-1- Small cell lung cancer	21.2	HPAC- Pancreatic adenocarcinoma	0.0
Colo-205- Colon cancer	33.7	MIA PaCa-2- Pancreatic carcinoma	0.0
KM12- Colon cancer	18.2	CFPAC-1- Pancreatic ductal adenocarcinoma	0.0
KM20L2- Colon cancer	0.1	PANC-1- Pancreatic epithelioid ductal carcinoma	0.1
NCI-H716- Colon cancer	69.3	T24- Bladder carcinma (transitional cell)	0.4
SW-48- Colon adenocarcinoma	43.2	5637- Bladder carcinoma	0.0
SW1116- Colon adenocarcinoma	0.0	HT-1197- Bladder carcinoma	0.0
LS 174T- Colon adenocarcinoma	0.5	UM-UC-3- Bladder carcinma (transitional cell)	0.0
SW-948- Colon adenocarcinoma	6.8	A204- Rhabdomyosarcoma	0.0
SW-480- Colon adenocarcinoma	3.7	HT-1080- Fibrosarcoma	0.0
NCI-SNU-5- Gastric carcinoma	0.0	MG-63- Osteosarcoma	0.0
KATO III- Gastric carcinoma	0.0	SK-LMS-1- Leiomyosarcoma (vulva)	0.0
NCI-SNU-16- Gastric carcinoma	0.0	SJRH30- Rhabdomyosarcoma (met to bone marrow)	1.2
NCI-SNU-1- Gastric carcinoma	15.4	A431- Epidermoid carcinoma	0.0
RF-1- Gastric adenocarcinoma	0.0	WM266-4- Melanoma	0.0
RF-48- Gastric adenocarcinoma	0.0	DU 145- Prostate carcinoma (brain metastasis)	0.0
MKN-45- Gastric carcinoma	6.5	MDA-MB-468- Breast adenocarcinoma	0.0
NCI-N87- Gastric carcinoma	0.0	SCC-4- Squamous cell carcinoma	0.0

		of tongue	
OVCAR-5- Ovarian carcinoma	0.0	SCC-9- Squamous cell carcinoma of tongue	0.0
RL95-2- Uterine carcinoma	0.0	SCC-15- Squamous cell carcinoma of tongue	0.0
HelaS3- Cervical adenocarcinoma	0.0	CAL 27- Squamous cell carcinoma of tongue	0.0

Table 26F. Panel 4D

Tissue Name	Rel. Exp.(%) Ag1438, Run 164183835	Tissue Name	Rel. Exp.(%) Ag1438, Run 164183835
Secondary Th1 act	0.0	HUVEC IL-1beta	0.1
Secondary Th2 act	0.0	HUVEC IFN gamma	0.5
Secondary Tr1 act	0.0	HUVEC TNF alpha + IFN gamma	0.3
Secondary Th1 rest	0.0	HUVEC TNF alpha + IL4	0.1
Secondary Th2 rest	0.0	HUVEC IL-11	0.2
Secondary Tr1 rest	0.0	Lung Microvascular EC none	8.3
Primary Th1 act	0.0	Lung Microvascular EC TNFalpha + IL-1beta	6.1
Primary Th2 act	0.1	Microvascular Dermal EC none	11.9
Primary Tr1 act	0.0	Microsvasular Dermal EC TNFalpha + IL-1beta	6.2
Primary Th1 rest	0.0	Bronchial epithelium TNFalpha + IL1beta	0.6
Primary Th2 rest	0.0	Small airway epithelium none	0.6
Primary Tr1 rest	0.0	Small airway epithelium TNFalpha + IL-1beta	2.9
CD45RA CD4 lymphocyte act	0.0	Coronery artery SMC rest	0.1
CD45RO CD4 lymphocyte act	0.0	Coronery artery SMC TNFalpha + IL-1beta	0.0
CD8 lymphocyte act	0.0	Astrocytes rest	0.0
Secondary CD8 lymphocyte rest	0.0	Astrocytes TNFalpha + IL-1beta	0.0
Secondary CD8 lymphocyte act	0.0	KU-812 (Basophil) rest	0.0
CD4 lymphocyte none	0.1	KU-812 (Basophil) PMA/ionomycin	0.0
2ry Th1/Th2/Tr1_anti-CD95 CH11	0.0	CCD1106 (Keratinocytes) none	0.0
LAK cells rest	0.1	CCD1106 (Keratinocytes) TNFalpha + IL-1beta	0.0
LAK cells IL-2	0.0	Liver cirrhosis	2.1
LAK cells IL-2+IL-12	0.0	Lupus kidney	0.2
LAK cells IL-2+IFN gamma	0.0	NCI-H292 none	0.0
LAK cells IL-2+ IL-18	0.0	NCI-H292 IL-4	0.0
LAK cells PMA/ionomycin	0.0	NCI-H292 IL-9	0.0
NK Cells IL-2 rest	0.0	NCI-H292 IL-13	0.0
Two Way MLR 3 day	0.0	NCI-H292 IFN gamma	0.0
Two Way MLR 5 day	0.0	HPAEC none	0.0

Two Way MLR 7 day	0.0	HPAEC TNF alpha + IL-1 beta	0.1
PBMC rest	0.0	Lung fibroblast none	0.0
PBMC PWM	0.0	Lung fibroblast TNF alpha + IL-1 beta	0.0
PBMC PHA-L	0.0	Lung fibroblast IL-4	0.1
Ramos (B cell) none	0.0	Lung fibroblast IL-9	0.0
Ramos (B cell) ionomycin	0.0	Lung fibroblast IL-13	0.0
B lymphocytes PWM	0.0	Lung fibroblast IFN gamma	0.0
B lymphocytes CD40L and IL-4	0.0	Dermal fibroblast CCD1070 rest	0.0
EOL-1 dbcAMP	0.0	Dermal fibroblast CCD1070 TNF alpha	0.0
EOL-1 dbcAMP PMA/ionomycin	0.0	Dermal fibroblast CCD1070 IL-1 beta	0.0
Dendritic cells none	0.0	Dermal fibroblast IFN gamma	0.0
Dendritic cells LPS	0.0	Dermal fibroblast IL-4	1.2
Dendritic cells anti-CD40	0.0	IBD Colitis 2	0.4
Monocytes rest	0.0	IBD Crohn's	2.1
Monocytes LPS	0.0	Colon	**100.0**
Macrophages rest	0.0	Lung	1.9
Macrophages LPS	0.0	Thymus	0.4
HUVEC none	0.2	Kidney	0.5
HUVEC starved	0.8		

Panel 1.2 Summary: Ag1438 Highest expression of the CG56010-01 gene is seen in breast cancer (CT=20.1), with significant expression also seen in a cluster of lung cancer cell lines. Thus, expression of this gene could be used to differentiate between these samples and other samples on this panel. Furthermore, therapeutic modulation of the expression or function of this gene product through the application of small molecules or monoclonal antibodies may be effective in the treatment of breast and lung cancers.

Panel 1.3D Summary: Ag1438 Two experiments with the same probe and primer set produce results that are in excellent agreement, with highest expression of the CG56010-01 gene in a breast cancer cell line (CTs=27). Significant expression is also seen in a cluster of lung and colon cancer cell lines. This expression is consistent with the expression in Panel 1.2. Thus, expression of this gene could be used to differentiate between these samples and other samples on this panel. Furthermore, therapeutic modulation of the expression or function of this gene product through the application of small molecules or monoclonal antibodies may be effective in the treatment of breast, colon and lung cancers.

There is also moderate expression of this gene in several endocrine/metabolic related tissues, including adrenal, adipose, GI tract, pancreas and thyroid. Therefore, therapeutic

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modulation of this gene and/or gene-product may be useful in the treatment of diseases that involve the above mentioned tissues.

Panel 2D Summary: Ag1438 Two experiments with the same probe and primer set produce results that are in excellent agreement, with highest expression of the CG56010-01 gene in a lung cancer cell line (CTs=22-24). Significant expression is also seen in samples derived from breast, gastric, bladder, and uterine cancers. Thus, expression of this gene could be used to differentiate between these samples and other samples on this panel. Furthermore, therapeutic targeting of this gene product with a monoclonal antibody is anticipated to limit or block the extent of tumor growth in Subsets of breast cancers, gastric carcinomas, uterian tumors, transitional cell carcinomas of the bladder and lung adenocarcinomas/squamous cell carcinomas. Based on this gene's homology to trefoil 3, restricted normal tissue distribution, and preferential expression in proliferative cell lines seen in the previous panels, this gene product provides an excellent opportunity for drug targeting.

References:

Taupin D, Pedersen J, Familari M, Cook G, Yeomans N, Giraud AS.Augmented intestinal trefoil factor (TFF3) and loss of pS2 (TFF1) expression precedes metaplastic differentiation of gastric epithelium. Lab Invest 2001 Mar;81(3):397-408

The trefoil peptides spasmolytic polypeptide (SP), intestinal trefoil factor (ITF), and pS2 show lineage-specific expression in the normal gut and are strongly induced after mucosal injury. We assessed the relationship between this induction and the development of the regenerative epithelial lineage over time in the rat stomach and verified these observations in the metaplastic and dysplastic human stomach. Antral or colonic ulcers were induced in Wistar rats by application of serosal acetic acid and tissues harvested 2 hours to 125 days later. Human endoscopic biopsies or gastric resection specimens were also assessed. Tissues were examined by radioimmunoassay, immunoblotting, or immunohistochemistry for ITF, SP, and transforming growth factor alpha (rat) or ITF and pS2 (human) expression. ITF and SP mRNA in antral ulcer margins was localized by in situ hybridization. ITF and SP peptide expression rose steadily in ulcer margins after 4 days, with the rise in ITF being more pronounced. By 40 days, several hundred-fold elevations in ITF levels were present, with a field effect in uninvolved mucosa. Hyperproliferative, elongated glands of undifferentiated cells expressing abundant trefoil peptides and acid sulfomucins were present after day 12 and persisted after ulcer healing. ITF mRNA was aberrantly expressed in basal and mid-regions of these regenerative glands. In contrast, transforming growth factor alpha peptide expression rose

promptly after injury then fell to baseline levels with healing. Seven months after injury, gastric atrophy, intestinal metaplasia, and severe dysplasia with conserved ITF expression were seen. ITF was also induced in human intestinal metaplasia and conserved in all gastric cancers, whereas expression of the gastric peptide pS2 was progressively reduced in the

5   progression from metaplasia to dysplasia. Persistent, selective overexpression of ITF, possibly acting in an autocrine fashion, is a feature of regeneration after antral ulceration, and may provide insight into the nature of metaplastic phenotypes arising from chronic gastric injury. The loss of pS2 expression in metaplasia and cancer supports a role for this protein in gastric tumor suppression.

10   PMID: 11310832

Efstathiou JA, Noda M, Rowan A, Dixon C, Chinery R, Jawhari A, Hattori T, Wright NA, Bodmer WF, Pignatelli M.Intestinal trefoil factor controls the expression of the adenomatous polyposis coli-catenin and the E-cadherin-catenin complexes in human colon carcinoma cells. Proc Natl Acad Sci U S A 1998 Mar 17;95(6):3122-7

15   Intestinal trefoil factor 3 (TFF3) is a member of the trefoil family of peptides, small molecules constitutively expressed in epithelial tissues, including the gastrointestinal tract. TFF3 has been shown to promote migration of intestinal epithelial cells in vitro and to enhance mucosal healing and epithelial restitution in vivo. In this study, we evaluated the effect of recombinant TFF3 (rTFF3) stimulation on the expression and cellular localization of the

20   epithelial (E)-cadherin-catenin complex, a prime mediator of Ca2+ dependent cell-cell adhesion, and the adenomatous polyposis coli (APC)-catenin complex in HT29, HCT116, and SW480 colorectal carcinoma cell lines. Stimulation by rTFF3 (10(-9) M and 10(-8) M) for 20-24 hr led to cell detachment and to a reduction in intercellular adhesion in HT29 and HCT116 cells. In both cell lines, E-cadherin expression was down-regulated. The expression of APC,

25   alpha-catenin and beta-catenin also was decreased in HT29 cells, with a translocation of APC into the nucleus. No change in either cell adhesion or in the expression of E-cadherin, the catenins, and APC was detected in SW480 cells. In addition, TFF3 induced DNA fragmentation and morphological changes characteristic of apoptosis in HT29. Tyrphostin, a competitive inhibitor of protein tyrosine kinases, inhibited the effects of TFF3. Our results

30   indicate that by perturbing the complexes between E-cadherin, beta-catenin, and associated proteins, TFF3 may modulate epithelial cell adhesion, migration, and survival.

PMID: 9501226

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Panel 3D Summary: Ag1438 Highest expression of the CG56010-01 gene is seen in a lung cancer cell line (CTs=26). Significant expression is also seen in a cluster of gastric and colon cancer cell lines. Thus, expression of this gene could be used to differentiate between these samples and other samples on this panel. Furthermore, therapeutic modulation of the expression or function of this gene product through the application of small molecules or monoclonal antibodies may be effective in the treatment of gastric, colon and lung cancers.

Panel 4D Summary: Ag1438 Highest expression of the CG56010-01 transcript is observed in colon (CT=28.1). This expression is expected considering the nature of the protein encoded by this transcript, the trefoil factor 3 (ref. 1). The expression of this transcript is down regulated in colon from patients suffering from either Crohn's or colitis, suggesting a role for this gene in the normal homeostasis of this tissue. Therefore, agonistic antibodies or protein therapeutics may be beneficial for the the restoration of the normal function of the colon mucosa in inflammatory diseases such as inflammatory bowel disease. Low expression of this transcript is also observed in the microvasculature of the lung and the dermis suggesting a role for this gene in the maintenance of the integrity of the microvasculature. Therefore, therapeutics designed for this putative protein could be beneficial for the treatment of diseases associated with damaged microvasculature including heart diseases or inflammatory diseases, such as psoriasis, asthma, and chronic obstructive pulmonary diseases.

References:

dos Santos Silva E, Ulrich M, Doring G, Botzenhart K, Gott P. Trefoil factor family domain peptides in the human respiratory tract.

Trefoil factor family domain peptides (TFF) are thought to be involved in mucosal epithelial restitution and wound healing of the gastrointestinal tract and are up-regulated in ulceration and in a variety of solid tumours. It was hypothesized that TFFs are also expressed on mucosal surfaces of the human respiratory tract. Lung tissue, nasal polyps, and sputum samples from seven patients with cystic fibrosis (CF), two with chronic and acute bronchitis, and non-dysplastic material from two cases of bronchial adenocarcinoma were analysed for TFF expression by immunohistochemistry, immunofluorescence, western blot and RT-PCR. Expression of TFF1 and TFF3 was observed in material from all patients. TFFs were localized in goblet and ciliated cells, as well as in some submucosal cells of tracheobronchial tissues and nasal polyps from normal and CF individuals. In sputa of patients with CF and with chronic or acute bronchitis, TFF1 and TFF3 were detected by western blotting. Freshly cultivated nasal epithelial cells transcribed and secreted TFFs and mucins, whereas nasal cells cultivated for 6

weeks still expressed mucins, but not TFFs. Secreted TFFs and mucins also bound to the surface of Staphylococcus aureus in infected CF airways. In conclusion, TFF1 and TFF3 are expressed and secreted in normal and inflamed airways. The association of TFFs with bacteria may contribute to the anti-microbial mucociliary defence system.

SEC3 (CG56015-01)

Expression of gene CG56015-01 was assessed using the primer-probe set Ag1360, described in Table 27A. Results of the RTQ-PCR runs are shown in Tables 27B, 27C, 27D, 27E and 27F.

Table 27A. Probe Name Ag1360

Primers	Sequences	Length	Start Position
Forward	5'-gagctcagaccgtgtctaggtt-3' (SEQ ID NO:165)	22	641
Probe	TET-5'-cctgggggtctcctgctcagctca-3'-TAMRA (SEQ ID NO:166)	23	679
Reverse	5'-gtcctctccagaaggctcttc-3' (SEQ ID:167)	21	702

Table 27B. Panel 1.2

Tissue Name	Rel. Exp.(%) Ag1360, Run 134774681	Tissue Name	Rel. Exp.(%) Ag1360, Run 134774681
Endothelial cells	0.1	Renal ca. 786-0	14.0
Heart (Fetal)	0.4	Renal ca. A498	35.4
Pancreas	2.3	Renal ca. RXF 393	5.0
Pancreatic ca. CAPAN 2	0.3	Renal ca. ACHN	11.6
Adrenal Gland	1.3	Renal ca. UO-31	75.8
Thyroid	0.2	Renal ca. TK-10	57.8
Salivary gland	32.8	Liver	1.4
Pituitary gland	0.2	Liver (fetal)	0.8
Brain (fetal)	0.0	Liver ca. (hepatoblast) HepG2	0.2
Brain (whole)	0.0	Lung	3.8
Brain (amygdala)	0.0	Lung (fetal)	6.6
Brain (cerebellum)	0.0	Lung ca. (small cell) LX-1	0.0
Brain (hippocampus)	0.0	Lung ca. (small cell) NCI-H69	0.0
Brain (thalamus)	0.0	Lung ca. (s.cell var.) SHP-77	0.0
Cerebral Cortex	0.1	Lung ca. (large cell)NCI-H460	0.0
Spinal cord	0.1	Lung ca. (non-sm. cell) A549	0.9
glio/astro U87-MG	0.0	Lung ca. (non-s.cell) NCI-H23	0.0
glio/astro U-118-MG	0.0	Lung ca. (non-s.cell) HOP-62	0.0
astrocytoma SW1783	0.0	Lung ca. (non-s.cl) NCI-H522	0.0

neuro*; met SK-N-AS	0.0	Lung ca. (squam.) SW 900	20.2
astrocytoma SF-539	0.0	Lung ca. (squam.) NCI-H596	0.0
astrocytoma SNB-75	0.0	Mammary gland	4.6
glioma SNB-19	0.0	Breast ca.* (pl.ef) MCF-7	0.0
glioma U251	0.1	Breast ca.* (pl.ef) MDA-MB-231	0.1
glioma SF-295	0.1	Breast ca.* (pl. ef) T47D	0.1
Heart	0.3	Breast ca. BT-549	0.0
Skeletal Muscle	0.1	Breast ca. MDA-N	0.0
Bone marrow	6.6	Ovary	0.2
Thymus	0.2	Ovarian ca. OVCAR-3	0.0
Spleen	0.2	Ovarian ca. OVCAR-4	2.2
Lymph node	0.0	Ovarian ca. OVCAR-5	5.7
Colorectal Tissue	1.4	Ovarian ca. OVCAR-8	0.9
Stomach	17.6	Ovarian ca. IGROV-1	31.6
Small intestine	3.7	Ovarian ca. (ascites) SK-OV-3	1.6
Colon ca. SW480	0.0	Uterus	13.2
Colon ca.* SW620 (SW480 met)	0.0	Placenta	0.6
Colon ca. HT29	2.9	Prostate	2.1
Colon ca. HCT-116	0.0	Prostate ca.* (bone met) PC-3	0.0
Colon ca. CaCo-2	0.1	Testis	0.2
Colon ca. Tissue (ODO3866)	3.8	Melanoma Hs688(A).T	0.0
Colon ca. HCC-2998	1.4	Melanoma* (met) Hs688(B).T	0.0
Gastric ca.* (liver met) NCI-N87	1.7	Melanoma UACC-62	0.0
Bladder	19.6	Melanoma M14	0.0
Trachea	7.2	Melanoma LOX IMVI	0.0
Kidney	**100.0**	Melanoma* (met) SK-MEL-5	0.0
Kidney (fetal)	21.0		

Table 27C. Panel 1.3D

Tissue Name	Rel. Exp.(%) Ag1360, Run 146124878	Tissue Name	Rel. Exp.(%) Ag1360, Run 146124878
Liver adenocarcinoma	0.0	Kidney (fetal)	28.1
Pancreas	3.9	Renal ca. 786-0	45.4
Pancreatic ca. CAPAN 2	1.4	Renal ca. A498	**100.0**
Adrenal gland	0.5	Renal ca. RXF 393	12.8
Thyroid	0.2	Renal ca. ACHN	61.1
Salivary gland	16.7	Renal ca. UO-31	25.5
Pituitary gland	0.1	Renal ca. TK-10	72.2
Brain (fetal)	0.0	Liver	1.3
Brain (whole)	0.0	Liver (fetal)	1.1
Brain (amygdala)	0.1	Liver ca. (hepatoblast) HepG2	0.3

Brain (cerebellum)	0.0	Lung	8.1
Brain (hippocampus)	0.0	Lung (fetal)	13.0
Brain (substantia nigra)	0.0	Lung ca. (small cell) LX-1	0.0
Brain (thalamus)	0.1	Lung ca. (small cell) NCI-H69	0.0
Cerebral Cortex	0.0	Lung ca. (s.cell var.) SHP-77	0.0
Spinal cord	0.4	Lung ca. (large cell)NCI-H460	0.0
glio/astro U87-MG	0.0	Lung ca. (non-sm. cell) A549	0.4
glio/astro U-118-MG	0.0	Lung ca. (non-s.cell) NCI-H23	0.0
astrocytoma SW1783	0.0	Lung ca. (non-s.cell) HOP-62	0.0
neuro*; met SK-N-AS	0.0	Lung ca. (non-s.cl) NCI-H522	0.0
astrocytoma SF-539	0.0	Lung ca. (squam.) SW 900	17.6
astrocytoma SNB-75	27.2	Lung ca. (squam.) NCI-H596	0.0
glioma SNB-19	0.0	Mammary gland	10.0
glioma U251	0.1	Breast ca.* (pl.ef) MCF-7	0.0
glioma SF-295	0.8	Breast ca.* (pl.ef) MDA-MB-231	0.7
Heart (fetal)	0.7	Breast ca.* (pl.ef) T47D	0.1
Heart	0.0	Breast ca. BT-549	0.0
Skeletal muscle (fetal)	0.2	Breast ca. MDA-N	0.0
Skeletal muscle	0.0	Ovary	0.5
Bone marrow	9.0	Ovarian ca. OVCAR-3	0.0
Thymus	0.2	Ovarian ca. OVCAR-4	1.9
Spleen	0.3	Ovarian ca. OVCAR-5	13.8
Lymph node	0.1	Ovarian ca. OVCAR-8	0.5
Colorectal	5.2	Ovarian ca. IGROV-1	36.6
Stomach	20.4	Ovarian ca.* (ascites) SK-OV-3	3.1
Small intestine	3.2	Uterus	0.5
Colon ca. SW480	0.0	Placenta	1.1
Colon ca.* SW620(SW480 met)	0.0	Prostate	1.2
Colon ca. HT29	3.5	Prostate ca.* (bone met)PC-3	1.2
Colon ca. HCT-116	0.0	Testis	0.4
Colon ca. CaCo-2	1.3	Melanoma Hs688(A).T	0.1
Colon ca. tissue(ODO3866)	34.6	Melanoma* (met) Hs688(B).T	0.0
Colon ca. HCC-2998	3.5	Melanoma UACC-62	0.0
Gastric ca.* (liver met) NCI-N87	11.0	Melanoma M14	0.0
Bladder	11.3	Melanoma LOX IMVI	0.0

| Trachea | 14.0 | Melanoma* (met) SK-MEL-5 | 0.0 |
| Kidney | **100.0** | Adipose | 2.4 |

Table 27D. Panel 2D

Tissue Name	Rel. Exp.(%) Ag1360, Run 145081245	Rel. Exp.(%) Ag1360, Run 145419838	Tissue Name	Rel. Exp.(%) Ag1360, Run 145081245	Rel. Exp.(%) Ag1360, Run 145419838
Normal Colon	0.6	0.3	Kidney Margin 8120608	**100.0**	**100.0**
CC Well to Mod Diff (ODO3866)	2.1	0.9	Kidney Cancer 8120613	0.1	0.0
CC Margin (ODO3866)	0.7	0.3	Kidney Margin 8120614	80.1	10.9
CC Gr.2 rectosigmoid (ODO3868)	1.8	1.0	Kidney Cancer 9010320	11.8	5.3
CC Margin (ODO3868)	0.1	0.0	Kidney Margin 9010321	84.7	49.0
CC Mod Diff (ODO3920)	2.3	0.8	Normal Uterus	0.0	0.0
CC Margin (ODO3920)	0.4	0.1	Uterus Cancer 064011	1.3	1.3
CC Gr.2 ascend colon (ODO3921)	2.8	0.7	Normal Thyroid	0.0	0.0
CC Margin (ODO3921)	0.6	0.1	Thyroid Cancer 064010	2.2	1.1
CC from Partial Hepatectomy (ODO4309) Mets	6.3	0.3	Thyroid Cancer A302152	2.9	1.9
Liver Margin (ODO4309)	0.0	0.0	Thyroid Margin A302153	0.0	0.0
Colon mets to lung (OD04451-01)	1.7	0.8	Normal Breast	1.4	0.4
Lung Margin (OD04451-02)	0.7	0.2	Breast Cancer (OD04566)	0.0	0.1
Normal Prostate 6546-1	0.3	0.3	Breast Cancer (OD04590-01)	0.1	0.0
Prostate Cancer (OD04410)	0.3	0.3	Breast Cancer Mets (OD04590-03)	0.0	0.0
Prostate Margin (OD04410)	0.3	0.4	Breast Cancer Metastasis (OD04655-05)	0.6	0.8
Prostate Cancer (OD04720-01)	0.3	0.4	Breast Cancer 064006	1.6	1.4
Prostate Margin (OD04720-02)	1.3	0.9	Breast Cancer 1024	2.0	0.9
Normal Lung 061010	0.6	0.2	Breast Cancer 9100266	1.3	1.1
Lung Met to Muscle (ODO4286)	0.0	0.0	Breast Margin 9100265	0.5	0.4
Muscle Margin (ODO4286)	0.0	0.0	Breast Cancer A209073	1.2	1.0
Lung Malignant	2.2	0.9	Breast Margin	1.6	0.3

Cancer (OD03126)			A2090734		
Lung Margin (OD03126)	1.4	0.4	Normal Liver	0.0	0.0
Lung Cancer (OD04404)	2.2	0.8	Liver Cancer 064003	0.1	0.0
Lung Margin (OD04404)	1.1	0.4	Liver Cancer 1025	0.0	0.0
Lung Cancer (OD04565)	0.5	0.7	Liver Cancer 1026	0.1	0.0
Lung Margin (OD04565)	1.0	1.8	Liver Cancer 6004-T	0.1	0.0
Lung Cancer (OD04237-01)	0.5	0.2	Liver Tissue 6004-N	0.0	0.0
Lung Margin (OD04237-02)	1.4	0.2	Liver Cancer 6005-T	0.1	0.1
Ocular Mel Met to Liver (ODO4310)	0.0	0.0	Liver Tissue 6005-N	0.0	0.0
Liver Margin (ODO4310)	0.1	0.1	Normal Bladder	5.0	6.5
Melanoma Mets to Lung (OD04321)	0.0	0.0	Bladder Cancer 1023	1.3	0.9
Lung Margin (OD04321)	1.4	0.5	Bladder Cancer A302173	0.5	0.2
Normal Kidney	45.4	14.9	Bladder Cancer (OD04718-01)	1.9	1.8
Kidney Ca, Nuclear grade 2 (OD04338)	44.1	19.6	Bladder Normal Adjacent (OD04718-03)	0.0	0.0
Kidney Margin (OD04338)	8.0	3.4	Normal Ovary	0.1	0.0
Kidney Ca Nuclear grade 1/2 (OD04339)	5.4	3.4	Ovarian Cancer 064008	4.1	2.3
Kidney Margin (OD04339)	83.5	99.3	Ovarian Cancer (OD04768-07)	20.7	13.5
Kidney Ca, Clear cell type (OD04340)	23.5	31.9	Ovary Margin (OD04768-08)	0.1	0.2
Kidney Margin (OD04340)	23.5	13.9	Normal Stomach	0.2	0.2
Kidney Ca, Nuclear grade 3 (OD04348)	0.3	0.2	Gastric Cancer 9060358	0.3	0.1
Kidney Margin (OD04348)	11.8	11.3	Stomach Margin 9060359	1.7	1.5
Kidney Cancer (OD04622-01)	16.6	18.2	Gastric Cancer 9060395	3.0	1.5
Kidney Margin (OD04622-03)	7.0	6.4	Stomach Margin 9060394	3.1	1.5
Kidney Cancer (OD04450-01)	36.3	25.9	Gastric Cancer 9060397	4.6	3.4
Kidney Margin (OD04450-03)	14.1	6.6	Stomach Margin 9060396	1.8	1.1
Kidney Cancer 8120607	13.5	14.0	Gastric Cancer 064005	1.7	1.0

Table 27E. Panel 3D

Tissue Name	Rel. Exp.(%) Ag1360, Run 170745274	Tissue Name	Rel. Exp.(%) Ag1360, Run 170745274
Daoy- Medulloblastoma	0.0	Ca Ski- Cervical epidermoid carcinoma (metastasis)	0.0
TE671- Medulloblastoma	0.0	ES-2- Ovarian clear cell carcinoma	0.0
D283 Med- Medulloblastoma	0.0	Ramos- Stimulated with PMA/ionomycin 6h	0.0
PFSK-1- Primitive Neuroectodermal	0.0	Ramos- Stimulated with PMA/ionomycin 14h	0.0
XF-498- CNS	0.0	MEG-01- Chronic myelogenous leukemia (megokaryoblast)	4.2
SNB-78- Glioma	0.8	Raji- Burkitt's lymphoma	0.0
SF-268- Glioblastoma	0.0	Daudi- Burkitt's lymphoma	0.0
T98G- Glioblastoma	0.0	U266- B-cell plasmacytoma	0.0
SK-N-SH- Neuroblastoma (metastasis)	0.0	CA46- Burkitt's lymphoma	0.0
SF-295- Glioblastoma	0.3	RL- non-Hodgkin's B-cell lymphoma	36.6
Cerebellum	0.0	JM1- pre-B-cell lymphoma	0.0
Cerebellum	0.2	Jurkat- T cell leukemia	1.4
NCI-H292- Mucoepidermoid lung carcinoma	8.4	TF-1- Erythroleukemia	5.6
DMS-114- Small cell lung cancer	0.0	HUT 78- T-cell lymphoma	0.0
DMS-79- Small cell lung cancer	0.0	U937- Histiocytic lymphoma	0.0
NCI-H146- Small cell lung cancer	0.0	KU-812- Myelogenous leukemia	20.6
NCI-H526- Small cell lung cancer	0.0	769-P- Clear cell renal carcinoma	73.7
NCI-N417- Small cell lung cancer	0.1	Caki-2- Clear cell renal carcinoma	**100.0**
NCI-H82- Small cell lung cancer	0.0	SW 839- Clear cell renal carcinoma	29.9
NCI-H157- Squamous cell lung cancer (metastasis)	0.0	G401- Wilms' tumor	0.0
NCI-H1155- Large cell lung cancer	1.0	Hs766T- Pancreatic carcinoma (LN metastasis)	2.6
NCI-H1299- Large cell lung cancer	0.0	CAPAN-1- Pancreatic adenocarcinoma (liver metastasis)	16.6
NCI-H727- Lung carcinoid	0.0	SU86.86- Pancreatic carcinoma (liver metastasis)	2.0
NCI-UMC-11- Lung carcinoid	0.0	BxPC-3- Pancreatic adenocarcinoma	1.6
LX-1- Small cell lung cancer	0.0	HPAC- Pancreatic adenocarcinoma	1.5
Colo-205- Colon cancer	3.0	MIA PaCa-2- Pancreatic carcinoma	0.0
KM12- Colon cancer	0.0	CFPAC-1- Pancreatic ductal adenocarcinoma	8.2
KM20L2- Colon cancer	9.6	PANC-1- Pancreatic epithelioid ductal carcinoma	0.0
NCI-H716- Colon cancer	0.0	T24- Bladder carcinma (transitional cell)	0.0

SW-48- Colon adenocarcinoma	26.2	5637- Bladder carcinoma	6.9
SW1116- Colon adenocarcinoma	0.0	HT-1197- Bladder carcinoma	0.0
LS 174T- Colon adenocarcinoma	23.0	UM-UC-3- Bladder carcinma (transitional cell)	0.0
SW-948- Colon adenocarcinoma	1.4	A204- Rhabdomyosarcoma	1.3
SW-480- Colon adenocarcinoma	6.7	HT-1080- Fibrosarcoma	0.0
NCI-SNU-5- Gastric carcinoma	0.0	MG-63- Osteosarcoma	0.0
KATO III- Gastric carcinoma	18.7	SK-LMS-1- Leiomyosarcoma (vulva)	0.0
NCI-SNU-16- Gastric carcinoma	0.0	SJRH30- Rhabdomyosarcoma (met to bone marrow)	0.0
NCI-SNU-1- Gastric carcinoma	4.5	A431- Epidermoid carcinoma	13.2
RF-1- Gastric adenocarcinoma	0.0	WM266-4- Melanoma	0.0
RF-48- Gastric adenocarcinoma	0.0	DU 145- Prostate carcinoma (brain metastasis)	0.0
MKN-45- Gastric carcinoma	2.0	MDA-MB-468- Breast adenocarcinoma	74.2
NCI-N87- Gastric carcinoma	1.7	SCC-4- Squamous cell carcinoma of tongue	0.0
OVCAR-5- Ovarian carcinoma	6.0	SCC-9- Squamous cell carcinoma of tongue	0.0
RL95-2- Uterine carcinoma	3.1	SCC-15- Squamous cell carcinoma of tongue	0.0
HelaS3- Cervical adenocarcinoma	0.0	CAL 27- Squamous cell carcinoma of tongue	16.6

Table 27F. Panel 4.1D

Tissue Name	Rel. Exp.(%) Ag1360, Run 170737092	Tissue Name	Rel. Exp.(%) Ag1360, Run 170737092
Secondary Th1 act	0.0	HUVEC IL-1beta	0.1
Secondary Th2 act	0.0	HUVEC IFN gamma	0.0
Secondary Tr1 act	0.0	HUVEC TNF alpha + IFN gamma	0.1
Secondary Th1 rest	0.0	HUVEC TNF alpha + IL4	0.1
Secondary Th2 rest	0.0	HUVEC IL-11	0.0
Secondary Tr1 rest	0.0	Lung Microvascular EC none	0.0
Primary Th1 act	0.0	Lung Microvascular EC TNFalpha + IL-1beta	0.0
Primary Th2 act	0.0	Microvascular Dermal EC none	0.0
Primary Tr1 act	0.0	Microvasular Dermal EC TNFalpha + IL-1beta	0.0
Primary Th1,rest	0.0	Bronchial epithelium TNFalpha + IL1beta	2.1
Primary Th2 rest	0.0	Small airway epithelium none	11.7
Primary Tr1 rest	0.0	Small airway epithelium TNFalpha + IL-1beta	13.0
CD45RA CD4 lymphocyte	0.0	Coronery artery SMC rest	0.5

act			
CD45RO CD4 lymphocyte act	0.0	Coronery artery SMC TNFalpha + IL-1beta	0.7
CD8 lymphocyte act	0.0	Astrocytes rest	0.0
Secondary CD8 lymphocyte rest	0.0	Astrocytes TNFalpha + IL-1beta	0.2
Secondary CD8 lymphocyte act	0.0	KU-812 (Basophil) rest	1.6
CD4 lymphocyte none	0.0	KU-812 (Basophil) PMA/ionomycin	0.9
2ry Th1/Th2/Tr1_anti-CD95 CH11	0.0	CCD1106 (Keratinocytes) none	0.0
LAK cells rest	0.0	CCD1106 (Keratinocytes) TNFalpha + IL-1beta	0.5
LAK cells IL-2	0.0	Liver cirrhosis	3.3
LAK cells IL-2+IL-12	0.0	NCI-H292 none	2.3
LAK cells IL-2+IFN gamma	0.0	NCI-H292 IL-4	4.4
LAK cells IL-2+ IL-18	0.0	NCI-H292 IL-9	1.8
LAK cells PMA/ionomycin	0.0	NCI-H292 IL-13	4.0
NK Cells IL-2 rest	0.0	NCI-H292 IFN gamma	2.7
Two Way MLR 3 day	0.0	HPAEC none	0.1
Two Way MLR 5 day	0.0	HPAEC TNF alpha + IL-1 beta	0.2
Two Way MLR 7 day	0.0	Lung fibroblast none	0.0
PBMC rest	0.1	Lung fibroblast TNF alpha + IL-1 beta	0.0
PBMC PWM	0.0	Lung fibroblast IL-4	0.0
PBMC PHA-L	0.0	Lung fibroblast IL-9	0.0
Ramos (B cell) none	0.0	Lung fibroblast IL-13	0.1
Ramos (B cell) ionomycin	0.0	Lung fibroblast IFN gamma	0.1
B lymphocytes PWM	0.0	Dermal fibroblast CCD1070 rest	0.0
B lymphocytes CD40L and IL-4	0.0	Dermal fibroblast CCD1070 TNF alpha	0.0
EOL-1 dbcAMP	0.0	Dermal fibroblast CCD1070 IL-1 beta	0.1
EOL-1 dbcAMP PMA/ionomycin	0.0	Dermal fibroblast IFN gamma	0.1
Dendritic cells none	0.0	Dermal fibroblast IL-4	0.0
Dendritic cells LPS	0.0	Dermal Fibroblasts rest	0.1
Dendritic cells anti-CD40	0.0	Neutrophils TNFa+LPS	0.1
Monocytes rest	0.0	Neutrophils rest	0.0
Monocytes LPS	0.0	Colon	0.6
Macrophages rest	0.0	Lung	2.0
Macrophages LPS	0.0	Thymus	2.8
HUVEC none	0.0	Kidney	**100.0**
HUVEC starved	0.0		

Panel 1.2 Summary: Ag1360 The expression of the CG56015-01 gene appears to be highest in a sample derived from normal kidney tissue (CT=20.7). In addition, there appears to

be substantial expression in kidney cancer derived cell lines, ovarian cancer derived cell lines, colon cancer derived cell lines and normal salivary gland. Thus, the expression of this gene could be used to distinguish normal kidney from the other samples in the panel. The product of this gene is hypothesized to be involved in cellular communication. Therefore, therapeutic

5   modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be beneficial in the treatment of kidney cancer, ovarian cancer or colon cancer.

This gene product also has a moderate to high level of expression in a number of endocrine/metabolically relevant tissues, including brain, GI tract, pituitary, and liver. Thus,

10   therapeutic modulation of this gene product may be useful in the treatment of metabolic disorders, such as obesity and diabetes.

References:

Kocher O, Cheresh P, Lee SW. Identification and partial characterization of a novel membrane-associated protein (MAP17) up-regulated in human carcinomas and modulating

15   cell replication and tumor growth. Am J Pathol. 1996 Aug;149(2):493-500.

Using the differential display technique, we have recently reported the identification of a novel gene originally designated DD96. As determined by Northern blot and in situ hybridization, DD96 was expressed at significant levels only in a single epithelial cell population, the proximal tubular epithelial cells of the kidney. However, it was diffusely

20   expressed in various carcinomas originating from kidney, colon, lung, and breast. Using a specific polyclonal antibody, we have not determined that the DD96 protein product is a 17-kd membrane-associated protein, which we have therefore redesignated MAP17. In normal tissues, MAP17 is expressed in significant amounts only in the kidney, where it was localized to the brush border of proximal tubular epithelial cells. However, MAP17 is expressed

25   abundantly in carcinomas arising from kidney, colon, lung, and breast, in some cases with a membrane-associated apical glandular distribution. In tissue culture, MAP17 was localized to the cell membrane in areas of cell-cell contact, ie, the distribution of cell-function-associated proteins. Transfection of a full-length wild-type DD96 cDNA clone into a colon carcinoma cell line, HT-29, markedly decreased cell proliferation in vitro and tumor growth in vivo.

30   Although the precise function of MAP17 remains to be determined, our findings suggest that this protein may play an important role in tumor biology.

Panel 1.3D Summary: Ag1360 The expression of the CG56015-01 gene appears to be highest in a sample derived from a renal cancer cell line (CT=26.5). In addition, there

appears to be substantial expression in other kidney cancer derived cell lines, ovarian cancer derived cell lines, colon cancer tissue, stomach tissue, an astrocytoma cell line a pancreatic cancer cell line and normal kidney and salivary gland. Thus, the expression of this gene could be used to distinguish normal kidney or A498 cells from the other samples in the panel.

Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, monoclonal antibodies or protein therapeutics may limit or block the extent of tumor cell growth and be beneficial in the treatment of kidney cancer, ovarian cancer or colon cancer.

Panel 2D Summary: Ag1360 The expression of the CG56015-01 gene was assessed in two independent runs in panel 2D with excellent concordance between the runs. Overall, the expression of this gene was highest in normal kidney tissue, a result in concordance with expression in Panels 1.2 and 1.3D. Thus, the expression of this gene could be used to distinguish normal kidney tissue from other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be beneficial in the treatment of kidney cancer.

Panel 3D Summary: Ag1360 The expression of this gene appears to be highest in a sample derived from a renal cancer cell line (Caki-2). In addition there is substantial expression associated with other kidney cancer cell lines, colon cnacer cell lines and a breast cancer cell line. Thus, the expression of this gene could be used to distinguish Caki-2 cells from other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be beneficial in the treatment of kidney cancer, breast cancer or colon cancer.

Panel 4.1D Summary: Ag1360 This transcript, encoding for a membrane associated epithelial protein, is highly expressed in kidney (CT 24.4). High expression of this transcript is also found in small airway and bronchial epithelium, (CT 27) and to a lower extent in the mucoepidermoid cell line H292 (CT 29). The protein encoded by this transcript appears to be involved in cell-cell communication and/or proliferation. Therefore modulation of the expression or activity of this putative protein by antibodies may be useful for the treatment of lung diseases associated with hyperplasia and/or activation of mucus producing cells such as asthma, chronic obstructive pulmonary diseases, emphysema and/or lung cancer

SEC12 (CG56035-01)

Expression of gene CG56035-01 was assessed using the primer-probe set Ag1390, described in Table 28A. Results of the RTQ-PCR runs are shown in Tables 28B, 28C, and 28D.

Table 28A. Probe Name Ag1390

Primers	Sequences	Length	Start Position
Forward	5'-cccacaagagaggtatgtcact-3' (SEQ ID NO:168)	22	2129
Probe	TET-5'-ttacttcccaggacatccaccctgag-3'-TAMRA (SEQ ID NO:169)	26	2155
Reverse	5'-aaaatttggcactcacatgaag-3' (SEQ ID NO:170)	22	2207

Table 28B. Panel 1.2

Tissue Name	Rel. Exp.(%) Ag1390, Run 134918864	Rel. Exp.(%) Ag1390, Run 138253152	Tissue Name	Rel. Exp.(%) Ag1390, Run 134918864	Rel. Exp.(%) Ag1390, Run 138253152
Endothelial cells	0.0	0.0	Renal ca. 786-0	0.5	0.2
Heart (Fetal)	0.2	0.4	Renal ca. A498	0.9	0.5
Pancreas	0.3	0.0	Renal ca. RXF 393	0.1	0.1
Pancreatic ca. CAPAN 2	0.0	0.0	Renal ca. ACHN	0.3	0.5
Adrenal Gland	7.2	0.1	Renal ca. UO-31	0.6	0.5
Thyroid	0.8	0.1	Renal ca. TK-10	0.5	0.4
Salivary gland	10.6	0.9	Liver	0.1	0.2
Pituitary gland	6.4	0.9	Liver (fetal)	0.0	0.0
Brain (fetal)	0.0	0.0	Liver ca. (hepatoblast) HepG2	1.2	0.4
Brain (whole)	1.1	0.0	Lung	0.5	0.2
Brain (amygdala)	0.2	0.1	Lung (fetal)	0.0	0.0
Brain (cerebellum)	0.3	0.1	Lung ca. (small cell) LX-1	0.0	0.0
Brain (hippocampus)	0.3	0.4	Lung ca. (small cell) NCI-H69	0.1	0.2
Brain (thalamus)	0.5	0.3	Lung ca. (s.cell var.) SHP-77	0.0	0.0
Cerebral Cortex	0.1	0.2	Lung ca. (large cell)NCI-H460	0.1	0.1
Spinal cord	1.2	0.5	Lung ca. (non-sm. cell) A549	0.3	0.3
glio/astro U87-MG	0.1	0.0	Lung ca. (non-s.cell) NCI-H23	0.0	0.0
glio/astro U-118-MG	19.1	2.1	Lung ca. (non-s.cell) HOP-62	0.0	0.0
astrocytoma SW1783	0.1	0.0	Lung ca. (non-s.cl) NCI-H522	**100.0**	**100.0**
neuro*; met SK-N-AS	0.1	0.0	Lung ca. (squam.) SW 900	0.0	0.0
astrocytoma SF-539	1.1	0.6	Lung ca. (squam.) NCI-H596	0.0	0.0
astrocytoma SNB-75	0.2	0.1	Mammary gland	36.1	2.9
glioma SNB-19	0.3	0.2	Breast ca.* (pl.ef) MCF-7	0.0	0.0
glioma U251	0.0	0.4	Breast ca.* (pl.ef) MDA-MB-231	0.0	0.0
glioma SF-295	0.1	0.2	Breast ca.* (pl.	0.2	0.1

			ef) T47D		
Heart	2.3	7.3	Breast ca. BT-549	0.0	0.0
Skeletal Muscle	2.5	7.2	Breast ca. MDA-N	0.0	0.1
Bone marrow	0.1	0.2	Ovary	3.8	6.9
Thymus	0.2	0.0	Ovarian ca. OVCAR-3	0.0	0.0
Spleen	3.8	0.7	Ovarian ca. OVCAR-4	0.0	0.0
Lymph node	0.5	0.1	Ovarian ca. OVCAR-5	1.1	0.8
Colorectal Tissue	0.0	0.0	Ovarian ca. OVCAR-8	0.3	0.1
Stomach	2.0	0.1	Ovarian ca. IGROV-1	0.0	0.0
Small intestine	3.0	1.1	Ovarian ca. (ascites) SK-OV-3	0.0	0.0
Colon ca. SW480	0.0	0.0	Uterus	17.2	10.6
Colon ca.* SW620 (SW480 met)	0.0	0.0	Placenta	0.1	0.0
Colon ca. HT29	0.0	0.0	Prostate	4.2	2.4
Colon ca. HCT-116	0.0	0.0	Prostate ca.* (bone met) PC-3	0.0	0.0
Colon ca. CaCo-2	0.0	0.0	Testis	1.1	0.1
Colon ca. Tissue (ODO3866)	3.4	1.0	Melanoma Hs688(A).T	0.1	0.1
Colon ca. HCC-2998	0.0	0.0	Melanoma* (met) Hs688(B).T	0.4	0.7
Gastric ca.* (liver met) NCI-N87	0.0	0.0	Melanoma UACC-62	0.0	0.0
Bladder	16.8	20.4	Melanoma M14	0.0	0.0
Trachea	1.7	0.5	Melanoma LOX IMVI	0.0	0.0
Kidney	4.0	2.3	Melanoma* (met) SK-MEL-5	0.0	0.0
Kidney (fetal)	0.0	0.1			

Table 28C. Panel 2D

Tissue Name	Rel. Exp.(%) Ag1390, Run 145710824	Rel. Exp.(%) Ag1390, Run 145928345	Tissue Name	Rel. Exp.(%) Ag1390, Run 145710824	Rel. Exp.(%) Ag1390, Run 145928345
Normal Colon	6.2	6.7	Kidney Margin 8120608	0.0	0.0
CC Well to Mod Diff (ODO3866)	7.3	12.3	Kidney Cancer 8120613	0.0	0.0
CC Margin (ODO3866)	0.4	0.0	Kidney Margin 8120614	0.3	0.3
CC Gr.2 rectosigmoid (ODO3868)	0.3	0.8	Kidney Cancer 9010320	2.0	1.1
CC Margin (ODO3868)	0.3	0.0	Kidney Margin 9010321	0.0	0.0

CC Mod Diff (ODO3920)	0.0	0.0	Normal Uterus	2.5	6.5
CC Margin (ODO3920)	0.6	0.4	Uterus Cancer 064011	12.6	11.5
CC Gr.2 ascend colon (ODO3921)	4.2	3.9	Normal Thyroid	20.3	22.1
CC Margin (ODO3921)	0.2	0.4	Thyroid Cancer 064010	0.0	0.0
CC from Partial Hepatectomy (ODO4309) Mets	1.3	1.5	Thyroid Cancer A302152	0.8	0.9
Liver Margin (ODO4309)	0.0	0.0	Thyroid Margin A302153	2.6	3.6
Colon mets to lung (OD04451-01)	0.4	0.2	Normal Breast	8.4	7.2
Lung Margin (OD04451-02)	0.0	0.6	Breast Cancer (OD04566)	0.3	0.2
Normal Prostate 6546-1	8.9	10.8	Breast Cancer (OD04590-01)	14.8	14.4
Prostate Cancer (OD04410)	11.3	13.6	Breast Cancer Mets (OD04590-03)	34.6	29.9
Prostate Margin (OD04410)	0.9	0.8	Breast Cancer Metastasis (OD04655-05)	3.7	2.3
Prostate Cancer (OD04720-01)	5.6	4.4	Breast Cancer 064006	7.7	6.8
Prostate Margin (OD04720-02)	6.3	8.2	Breast Cancer 1024	7.7	9.3
Normal Lung 061010	5.9	6.3	Breast Cancer 9100266	6.4	6.7
Lung Met to Muscle (ODO4286)	0.0	0.1	Breast Margin 9100265	3.7	5.8
Muscle Margin (ODO4286)	3.9	14.4	Breast Cancer A209073	6.6	9.5
Lung Malignant Cancer (OD03126)	4.9	7.9	Breast Margin A2090734	3.4	1.5
Lung Margin (OD03126)	0.6	2.1	Normal Liver	0.0	0.0
Lung Cancer (OD04404)	3.1	3.8	Liver Cancer 064003	0.1	0.2
Lung Margin (OD04404)	3.5	7.3	Liver Cancer 1025	0.0	0.0
Lung Cancer (OD04565)	3.4	1.6	Liver Cancer 1026	1.0	1.1
Lung Margin (OD04565)	0.4	0.3	Liver Cancer 6004-T	0.5	0.0
Lung Cancer (OD04237-01)	5.0	3.7	Liver Tissue 6004-N	1.7	1.3
Lung Margin (OD04237-02)	2.0	4.3	Liver Cancer 6005-T	0.7	0.2
Ocular Mel Met to Liver (ODO4310)	0.2	0.0	Liver Tissue 6005-N	0.0	0.0
Liver Margin (ODO4310)	0.0	0.1	Normal Bladder	23.2	20.9

Melanoma Mets to Lung (OD04321)	0.0	0.2	Bladder Cancer 1023	2.7	2.1
Lung Margin (OD04321)	1.4	1.2	Bladder Cancer A302173	14.1	11.5
Normal Kidney	0.0	1.2	Bladder Cancer (OD04718-01)	1.7	1.4
Kidney Ca, Nuclear grade 2 (OD04338)	0.4	0.4	Bladder Normal Adjacent (OD04718-03)	12.1	12.8
Kidney Margin (OD04338)	0.3	0.5	Normal Ovary	5.9	5.1
Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	0.2	Ovarian Cancer 064008	**100.0**	**100.0**
Kidney Margin (OD04339)	0.7	0.8	Ovarian Cancer (OD04768-07)	1.7	4.1
Kidney Ca, Clear cell type (OD04340)	0.2	0.5	Ovary Margin (OD04768-08)	7.1	6.7
Kidney Margin (OD04340)	0.8	2.3	Normal Stomach	1.6	1.5
Kidney Ca, Nuclear grade 3 (OD04348)	0.0	0.3	Gastric Cancer 9060358	1.3	0.8
Kidney Margin (OD04348)	0.5	0.2	Stomach Margin 9060359	0.0	0.2
Kidney Cancer (OD04622-01)	0.3	0.2	Gastric Cancer 9060395	10.1	13.0
Kidney Margin (OD04622-03)	0.0	0.0	Stomach Margin 9060394	1.8	1.3
Kidney Cancer (OD04450-01)	0.3	0.0	Gastric Cancer 9060397	18.3	24.8
Kidney Margin (OD04450-03)	0.0	0.2	Stomach Margin 9060396	0.0	0.0
Kidney Cancer 8120607	3.3	3.3	Gastric Cancer 064005	3.1	3.9

Table 28D. Panel 4D

Tissue Name	Rel. Exp.(%) Ag1390, Run 162674334	Tissue Name	Rel. Exp.(%) Ag1390, Run 162674334
Secondary Th1 act	0.0	HUVEC IL-1beta	0.0
Secondary Th2 act	0.0	HUVEC IFN gamma	0.0
Secondary Tr1 act	0.0	HUVEC TNF alpha + IFN gamma	0.9
Secondary Th1 rest	0.0	HUVEC TNF alpha + IL4	0.0
Secondary Th2 rest	**100.0**	HUVEC IL-11	0.0
Secondary Tr1 rest	0.0	Lung Microvascular EC none	0.0
Primary Th1 act	0.0	Lung Microvascular EC TNFalpha + IL-1beta	0.0
Primary Th2 act	0.0	Microvascular Dermal EC none	0.0
Primary Tr1 act	0.0	Microsvasular Dermal EC TNFalpha + IL-1beta	0.0
Primary Th1 rest	0.0	Bronchial epithelium TNFalpha + IL1beta	0.0
Primary Th2 rest	0.0	Small airway epithelium none	0.0
Primary Tr1 rest	0.0	Small airway epithelium	0.0

		TNFalpha + IL-1beta	
CD45RA CD4 lymphocyte act	0.0	Coronery artery SMC rest	27.5
CD45RO CD4 lymphocyte act	0.0	Coronery artery SMC TNFalpha + IL-1beta	10.5
CD8 lymphocyte act	0.0	Astrocytes rest	8.0
Secondary CD8 lymphocyte rest	0.0	Astrocytes TNFalpha + IL-1beta	36.9
Secondary CD8 lymphocyte act	0.7	KU-812 (Basophil) rest	0.0
CD4 lymphocyte none	0.0	KU-812 (Basophil) PMA/ionomycin	0.0
2ry Th1/Th2/Tr1_anti-CD95 CH11	0.0	CCD1106 (Keratinocytes) none	0.0
LAK cells rest	0.0	CCD1106 (Keratinocytes) TNFalpha + IL-1beta	0.0
LAK cells IL-2	0.0	Liver cirrhosis	1.2
LAK cells IL-2+IL-12	1.8	Lupus kidney	0.8
LAK cells IL-2+IFN gamma	0.0	NCI-H292 none	0.0
LAK cells IL-2+ IL-18	1.3	NCI-H292 IL-4	0.0
LAK cells PMA/ionomycin	0.0	NCI-H292 IL-9	0.0
NK Cells IL-2 rest	1.8	NCI-H292 IL-13	0.0
Two Way MLR 3 day	0.7	NCI-H292 IFN gamma	0.0
Two Way MLR 5 day	0.0	HPAEC none	0.0
Two Way MLR 7 day	0.0	HPAEC TNF alpha + IL-1 beta	0.0
PBMC rest	1.1	Lung fibroblast none	0.7
PBMC PWM	0.0	Lung fibroblast TNF alpha + IL-1 beta	0.0
PBMC PHA-L	0.0	Lung fibroblast IL-4	2.5
Ramos (B cell) none	0.0	Lung fibroblast IL-9	1.3
Ramos (B cell) ionomycin	0.0	Lung fibroblast IL-13	1.6
B lymphocytes PWM	0.0	Lung fibroblast IFN gamma	1.3
B lymphocytes CD40L and IL-4	0.0	Dermal fibroblast CCD1070 rest	0.0
EOL-1 dbcAMP	0.0	Dermal fibroblast CCD1070 TNF alpha	0.0
EOL-1 dbcAMP PMA/ionomycin	0.8	Dermal fibroblast CCD1070 IL-1 beta	0.0
Dendritic cells none	1.7	Dermal fibroblast IFN gamma	0.0
Dendritic cells LPS	3.2	Dermal fibroblast IL-4	0.0
Dendritic cells anti-CD40	1.2	IBD Colitis 2	0.0
Monocytes rest	1.6	IBD Crohn's	0.0
Monocytes LPS	0.0	Colon	1.4
Macrophages rest	1.9	Lung	32.8
Macrophages LPS	0.0	Thymus	7.6
HUVEC none	0.0	Kidney	10.7
HUVEC starved	0.0		

AI_comprehensive panel_v1.0 Summary: Ag1390 Results from one experiment with the CG56035-01 gene are not included. The amp plot indicates that there were experimental difficulties with this run.

CNS_neurodegeneration_v1.0 Summary: Ag1390 Expression of the CG56035-01 gene is low/undetectable (CTs > 35) across all of the samples on this panel (data not shown).

Panel 1.2 Summary: Ag1390 The expression of the CG56035-01 gene was assessed in two independent runs in panel 1.2 with good concordance between runs. The expression of this gene appears to be highest in a sample derived from a lung cancer cell line (NCI-H522)(CTs=24). Thus, the expression of this gene could be used to distinguish NCI-H522 cells from other samples in the panel. Frizzled 4 genes, to which this gene is a homolog, act as soluble modulators of Wnt signaling. The WNT signaling cascade is involved in regulation of cytoskeletal rearrangements, apoptosis, and proliferation. Therefore, therapeutic modulation of this gene, through the use of small molecule drugs, protein therapeutics or antibodies might be beneficial in the treatment of lung cancer.

The pattern of expression of this isoform of this gene indicates that it may also have an important function in endocrine/metabolic physiology. Moderate to high levels of expression can be found in adrenal, brain, GI tract, pancreas, pituitary and thyroid. Thus, this gene product may be involved in the diagnosis and/or treatment of metabolic disorders, including obesity and diabetes.

In addition, higher levels of expression of this gene in lung (CTs=32-33)and liver (CTs=31-32) than in fetal lung and liver (CTs=36) suggest that it can also be used to differentiate between the adult and fetal forms of lung and liver.

Panel 2D Summary: Ag1390 The expression of the CG56035-01 gene was assessed in two independent runs in panel 2D with excellent concordance between runs. The expression of this gene appears to be highest in a sample derived from an ovarian cancer (CTs=30). In addition, there appears to be substantial expression associated with breast cancer. Thus, the expression of this gene could be used to distinguish this ovarian cancer sample from other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, protein therapeutics or antibodies might be beneficial in the treatment of breast or ovarian cancer.

Panel 3D Summary: Ag1390 Expression of the CG56035-01 gene is low/undetectable (CTs > 35) across all of the samples on this panel (data not shown).

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Panel 4D Summary: Ag1390 Highest expression of the CG56035-01 transcript is found in secondary Th2 rest cells (CT=31.7), but is absent in other T cells. Expression of this transcript is also found in the lung. This transcript encodes for a secreted frizzled related protein that are reported to antagonize the WNT/frizzled pathway. Since lung inflammatory diseases such as asthma and chronic obstructive pulmonary diseases are mediated by Th2 cells, this protein may be involved in the lung pathology associated with these Th2 T cells. Therefore, therapeutics designed against the protein encoded by this gene may be useful for the treatment of lung inflammatory diseases. This transcript is also expressed in astrocytes treated with TNF-a and IL-1 indicating that therapeutics designed against the protein encoded by this gene may be useful for the treatment of inflammatory CNS diseases such as multiple sclerosis.

SEC5 (CG56153-01)

Expression of gene CG56153-01 was assessed using the primer-probe set Ag1749, described in Table 29A. Results of the RTQ-PCR runs are shown in Tables 29B, 29C, 29D, 29E, 29F and 29G.

Table 29A. Probe Name Ag1749

Primers	Sequences	Length	Start Position
Forward	5'-ttactgggtaggattcgctttt-3' (SEQ ID NO:171)	22	216
Probe	TET-5'-aaatcctccagggacacagcccatt-3'-TAMRA (SEQ ID NO:172)	25	240
Reverse	5'-gggagtacctgaacacctcact-3' (SEQ ID NO:173)	22	271

Table 29B. CNS_neurodegeneration_v1.0

Tissue Name	Rel. Exp.(%) Ag1749, Run 207625049	Tissue Name	Rel. Exp.(%) Ag1749, Run 207625049
AD 1 Hippo	27.4	Control (Path) 3 Temporal Ctx	2.5
AD 2 Hippo	86.5	Control (Path) 4 Temporal Ctx	11.1
AD 3 Hippo	14.2	AD 1 Occipital Ctx	5.3
AD 4 Hippo	26.4	AD 2 Occipital Ctx (Missing)	0.0
AD 5 hippo	28.9	AD 3 Occipital Ctx	2.6
AD 6 Hippo	72.2	AD 4 Occipital Ctx	14.5
Control 2 Hippo	100.0	AD 5 Occipital Ctx	1.6
Control 4 Hippo	43.5	AD 6 Occipital Ctx	100.0
Control (Path) 3 Hippo	9.3	Control 1 Occipital Ctx	2.5
AD 1 Temporal Ctx	2.9	Control 2 Occipital Ctx	59.9
AD 2 Temporal Ctx	24.7	Control 3 Occipital Ctx	6.7
AD 3 Temporal Ctx	1.6	Control 4 Occipital Ctx	7.2
AD 4 Temporal Ctx	11.3	Control (Path) 1 Occipital Ctx	81.8
AD 5 Inf Temporal Ctx	23.0	Control (Path) 2 Occipital Ctx	10.6

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AD 5 SupTemporal Ctx	56.6	Control (Path) 3 Occipital Ctx	0.7
AD 6 Inf Temporal Ctx	12.3	Control (Path) 4 Occipital Ctx	11.1
AD 6 Sup Temporal Ctx	10.3	Control 1 Parietal Ctx	5.4
Control 1 Temporal Ctx	3.7	Control 2 Parietal Ctx	12.2
Control 2 Temporal Ctx	55.1	Control 3 Parietal Ctx	12.2
Control 3 Temporal Ctx	14.2	Control (Path) 1 Parietal Ctx	52.5
Control 4 Temporal Ctx	4.2	Control (Path) 2 Parietal Ctx	43.5
Control (Path) 1 Temporal Ctx	50.0	Control (Path) 3 Parietal Ctx	3.2
Control (Path) 2 Temporal Ctx	31.0	Control (Path) 4 Parietal Ctx	25.9

Table 29C. Panel 1.3D

Tissue Name	Rel. Exp.(%) Ag1749, Run 152485756	Tissue Name	Rel. Exp.(%) Ag1749, Run 152485756
Liver adenocarcinoma	0.0	Kidney (fetal)	0.4
Pancreas	0.0	Renal ca. 786-0	0.0
Pancreatic ca. CAPAN 2	0.0	Renal ca. A498	0.0
Adrenal gland	0.2	Renal ca. RXF 393	0.0
Thyroid	0.0	Renal ca. ACHN	0.0
Salivary gland	0.1	Renal ca. UO-31	0.0
Pituitary gland	**100.0**	Renal ca. TK-10	0.0
Brain (fetal)	13.5	Liver	0.0
Brain (whole)	4.4	Liver (fetal)	0.0
Brain (amygdala)	15.9	Liver ca. (hepatoblast) HepG2	0.0
Brain (cerebellum)	0.8	Lung	0.0
Brain (hippocampus)	21.3	Lung (fetal)	3.5
Brain (substantia nigra)	0.7	Lung ca. (small cell) LX-1	0.0
Brain (thalamus)	2.2	Lung ca. (small cell) NCI-H69	0.0
Cerebral Cortex	12.5	Lung ca. (s.cell var.) SHP-77	0.0
Spinal cord	3.3	Lung ca. (large cell)NCI-H460	0.0
glio/astro U87-MG	0.0	Lung ca. (non-sm. cell) A549	0.0
glio/astro U-118-MG	0.0	Lung ca. (non-s.cell) NCI-H23	0.0
astrocytoma SW1783	0.0	Lung ca. (non-s.cell) HOP-62	0.0
neuro*; met SK-N-AS	0.0	Lung ca. (non-s.cl) NCI-H522	0.0
astrocytoma SF-539	0.0	Lung ca. (squam.) SW 900	0.0
astrocytoma SNB-75	0.0	Lung ca. (squam.) NCI-H596	0.0
glioma SNB-19	0.0	Mammary gland	2.5

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glioma U251	0.0	Breast ca.* (pl.ef) MCF-7	0.0
glioma SF-295	0.0	Breast ca.* (pl.ef) MDA-MB-231	0.0
Heart (fetal)	0.8	Breast ca.* (pl.ef) T47D	0.0
Heart	0.0	Breast ca. BT-549	0.0
Skeletal muscle (fetal)	23.2	Breast ca. MDA-N	0.0
Skeletal muscle	0.0	Ovary	1.6
Bone marrow	0.0	Ovarian ca. OVCAR-3	0.0
Thymus	1.5	Ovarian ca. OVCAR-4	0.0
Spleen	0.1	Ovarian ca. OVCAR-5	0.0
Lymph node	0.2	Ovarian ca. OVCAR-8	0.0
Colorectal	2.9	Ovarian ca. IGROV-1	0.0
Stomach	0.4	Ovarian ca.* (ascites) SK-OV-3	0.0
Small intestine	1.5	Uterus	0.0
Colon ca. SW480	0.0	Placenta	18.7
Colon ca.* SW620(SW480 met)	0.0	Prostate	0.0
Colon ca. HT29	0.0	Prostate ca.* (bone met)PC-3	0.1
Colon ca. HCT-116	0.0	Testis	1.2
Colon ca. CaCo-2	0.0	Melanoma Hs688(A).T	0.0
Colon ca. tissue(ODO3866)	0.2	Melanoma* (met) Hs688(B).T	0.0
Colon ca. HCC-2998	0.0	Melanoma UACC-62	0.0
Gastric ca.* (liver met) NCI-N87	0.0	Melanoma M14	0.0
Bladder	0.0	Melanoma LOX IMVI	0.0
Trachea	0.1	Melanoma* (met) SK-MEL-5	0.0
Kidney	0.0	Adipose	1.4

Table 29D. Panel 2D

Tissue Name	Rel. Exp.(%) Ag1749, Run 152685549	Tissue Name	Rel. Exp.(%) Ag1749, Run 152685549
Normal Colon	32.5	Kidney Margin 8120608	0.0
CC Well to Mod Diff (ODO3866)	9.6	Kidney Cancer 8120613	0.0
CC Margin (ODO3866)	38.4	Kidney Margin 8120614	2.3
CC Gr.2 rectosigmoid (ODO3868)	13.5	Kidney Cancer 9010320	0.0
CC Margin (ODO3868)	78.5	Kidney Margin 9010321	0.0
CC Mod Diff (ODO3920)	0.0	Normal Uterus	0.0
CC Margin (ODO3920)	64.6	Uterus Cancer 064011	3.5
CC Gr.2 ascend colon (ODO3921)	6.8	Normal Thyroid	6.9
CC Margin (ODO3921)	34.6	Thyroid Cancer 064010	0.0
CC from Partial Hepatectomy (ODO4309) Mets	0.0	Thyroid Cancer A302152	0.0
Liver Margin (ODO4309)	0.0	Thyroid Margin A302153	2.1
Colon mets to lung (OD04451-	0.0	Normal Breast	51.1

01)			
Lung Margin (OD04451-02)	0.0	Breast Cancer (OD04566)	2.8
Normal Prostate 6546-1	11.7	Breast Cancer (OD04590-01)	12.0
Prostate Cancer (OD04410)	9.9	Breast Cancer Mets (OD04590-03)	74.2
Prostate Margin (OD04410)	11.7	Breast Cancer Metastasis (OD04655-05)	6.8
Prostate Cancer (OD04720-01)	5.6	Breast Cancer 064006	2.8
Prostate Margin (OD04720-02)	16.6	Breast Cancer 1024	8.1
Normal Lung 061010	6.2	Breast Cancer 9100266	2.3
Lung Met to Muscle (ODO4286)	0.0	Breast Margin 9100265	15.2
Muscle Margin (ODO4286)	8.7	Breast Cancer A209073	3.7
Lung Malignant Cancer (OD03126)	7.3	Breast Margin A2090734	9.1
Lung Margin (OD03126)	5.4	Normal Liver	0.0
Lung Cancer (OD04404)	4.3	Liver Cancer 064003	0.0
Lung Margin (OD04404)	0.0	Liver Cancer 1025	0.0
Lung Cancer (OD04565)	6.0	Liver Cancer 1026	0.0
Lung Margin (OD04565)	9.2	Liver Cancer 6004-T	0.0
Lung Cancer (OD04237-01)	0.0	Liver Tissue 6004-N	0.0
Lung Margin (OD04237-02)	1.4	Liver Cancer 6005-T	0.0
Ocular Mel Met to Liver (ODO4310)	0.0	Liver Tissue 6005-N	0.0
Liver Margin (ODO4310)	0.0	Normal Bladder	0.0
Melanoma Mets to Lung (OD04321)	0.0	Bladder Cancer 1023	1.9
Lung Margin (OD04321)	0.0	Bladder Cancer A302173	0.0
Normal Kidney	2.4	Bladder Cancer (OD04718-01)	3.3
Kidney Ca, Nuclear grade 2 (OD04338)	0.0	Bladder Normal Adjacent (OD04718-03)	**100.0**
Kidney Margin (OD04338)	0.0	Normal Ovary	12.2
Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	Ovarian Cancer 064008	4.0
Kidney Margin (OD04339)	0.0	Ovarian Cancer (OD04768-07)	0.0
Kidney Ca, Clear cell type (OD04340)	0.0	Ovary Margin (OD04768-08)	5.8
Kidney Margin (OD04340)	0.0	Normal Stomach	95.9
Kidney Ca, Nuclear grade 3 (OD04348)	0.0	Gastric Cancer 9060358	49.0
Kidney Margin (OD04348)	0.0	Stomach Margin 9060359	16.4
Kidney Cancer (OD04622-01)	0.0	Gastric Cancer 9060395	13.1
Kidney Margin (OD04622-03)	0.0	Stomach Margin 9060394	9.7
Kidney Cancer (OD04450-01)	0.0	Gastric Cancer 9060397	0.0
Kidney Margin (OD04450-03)	0.0	Stomach Margin 9060396	9.8
Kidney Cancer 8120607	0.0	Gastric Cancer 064005	29.9

Table 29E. Panel 4D

Tissue Name	Rel. Exp.(%) Ag1749,	Tissue Name	Rel. Exp.(%) Ag1749,

	Run 152685550		Run 152685550
Secondary Th1 act	9.2	HUVEC IL-1beta	15.3
Secondary Th2 act	6.7	HUVEC IFN gamma	90.1
Secondary Tr1 act	2.3	HUVEC TNF alpha + IFN gamma	13.3
Secondary Th1 rest	0.0	HUVEC TNF alpha + IL4	21.5
Secondary Th2 rest	0.0	HUVEC IL-11	37.1
Secondary Tr1 rest	0.8	Lung Microvascular EC none	0.0
Primary Th1 act	0.0	Lung Microvascular EC TNFalpha + IL-1beta	0.0
Primary Th2 act	0.0	Microvascular Dermal EC none	4.1
Primary Tr1 act	0.0	Microsvasular Dermal EC TNFalpha + IL-1beta	3.4
Primary Th1 rest	3.2	Bronchial epithelium TNFalpha + IL1beta	0.0
Primary Th2 rest	4.4	Small airway epithelium none	0.0
Primary Tr1 rest	0.0	Small airway epithelium TNFalpha + IL-1beta	0.0
CD45RA CD4 lymphocyte act	3.3	Coronery artery SMC rest	0.0
CD45RO CD4 lymphocyte act	5.6	Coronery artery SMC TNFalpha + IL-1beta	0.0
CD8 lymphocyte act	5.9	Astrocytes rest	78.5
Secondary CD8 lymphocyte rest	4.2	Astrocytes TNFalpha + IL-1beta	24.8
Secondary CD8 lymphocyte act	1.2	KU-812 (Basophil) rest	0.0
CD4 lymphocyte none	0.0	KU-812 (Basophil) PMA/ionomycin	0.0
2ry Th1/Th2/Tr1_anti-CD95 CH11	0.0	CCD1106 (Keratinocytes) none	0.0
LAK cells rest	0.0	CCD1106 (Keratinocytes) TNFalpha + IL-1beta	0.0
LAK cells IL-2	0.0	Liver cirrhosis	0.0
LAK cells IL-2+IL-12	2.0	Lupus kidney	0.0
LAK cells IL-2+IFN gamma	0.0	NCI-H292 none	0.0
LAK cells IL-2+ IL-18	0.0	NCI-H292 IL-4	0.0
LAK cells PMA/ionomycin	0.0	NCI-H292 IL-9	1.1
NK Cells IL-2 rest	2.0	NCI-H292 IL-13	0.0
Two Way MLR 3 day	0.0	NCI-H292 IFN gamma	0.8
Two Way MLR 5 day	1.2	HPAEC none	0.0
Two Way MLR 7 day	3.8	HPAEC TNF alpha + IL-1 beta	0.0
PBMC rest	0.0	Lung fibroblast none	2.1
PBMC PWM	3.3	Lung fibroblast TNF alpha + IL-1 beta	2.3
PBMC PHA-L	6.1	Lung fibroblast IL-4	1.6
Ramos (B cell) none	0.0	Lung fibroblast IL-9	2.0
Ramos (B cell) ionomycin	0.0	Lung fibroblast IL-13	1.4
B lymphocytes PWM	16.6	Lung fibroblast IFN gamma	3.7
B lymphocytes CD40L	1.0	Dermal fibroblast CCD1070 rest	0.0

and IL-4			
EOL-1 dbcAMP	0.0	Dermal fibroblast CCD1070 TNF alpha	0.0
EOL-1 dbcAMP PMA/ionomycin	0.0	Dermal fibroblast CCD1070 IL-1 beta	2.3
Dendritic cells none	0.0	Dermal fibroblast IFN gamma	0.0
Dendritic cells LPS	0.0	Dermal fibroblast IL-4	0.0
Dendritic cells anti-CD40	0.0	IBD Colitis 2	17.2
Monocytes rest	0.0	IBD Crohn's	0.9
Monocytes LPS	0.0	Colon	29.9
Macrophages rest	4.5	Lung	1.1
Macrophages LPS	2.2	Thymus	0.0
HUVEC none	58.2	Kidney	57.8
HUVEC starved	**100.0**		

Table 29F. Panel 5 Islet

Tissue Name	Rel. Exp.(%) Ag1749, Run 172371514	Tissue Name	Rel. Exp.(%) Ag1749, Run 172371514
97457_Patient-02go_adipose	3.5	94709_Donor 2 AM - A_adipose	0.0
97476_Patient-07sk_skeletal muscle	1.1	94710_Donor 2 AM - B_adipose	0.0
97477_Patient-07ut_uterus	0.0	94711_Donor 2 AM - C_adipose	0.0
97478_Patient-07pl_placenta	**100.0**	94712_Donor 2 AD - A_adipose	0.0
99167_Bayer Patient 1	1.2	94713_Donor 2 AD - B_adipose	0.0
97482_Patient-08ut_uterus	5.1	94714_Donor 2 AD - C_adipose	0.0
97483_Patient-08pl_placenta	13.5	94742_Donor 3 U - A_Mesenchymal Stem Cells	0.0
97486_Patient-09sk_skeletal muscle	0.6	94743_Donor 3 U - B_Mesenchymal Stem Cells	0.0
97487_Patient-09ut_uterus	0.0	94730_Donor 3 AM - A_adipose	0.0
97488_Patient-09pl_placenta	28.7	94731_Donor 3 AM - B_adipose	0.0
97492_Patient-10ut_uterus	1.0	94732_Donor 3 AM - C_adipose	0.0
97493_Patient-10pl_placenta	69.7	94733_Donor 3 AD - A_adipose	0.0
97495_Patient-11go_adipose	0.0	94734_Donor 3 AD - B_adipose	0.0
97496_Patient-11sk_skeletal muscle	0.0	94735_Donor 3 AD - C_adipose	0.0
97497_Patient-11ut_uterus	0.0	77138_Liver_HepG2untreated	0.0
97498_Patient-11pl_placenta	24.3	73556_Heart_Cardiac stromal cells (primary)	1.2
97500_Patient-12go_adipose	3.3	81735_Small Intestine	2.5
97501_Patient-12sk_skeletal muscle	0.5	72409_Kidney_Proximal Convoluted Tubule	0.0
97502_Patient-12ut_uterus	0.0	82685_Small intestine_Duodenum	0.0
97503_Patient-12pl_placenta	18.3	90650_Adrenal_Adrenocortical adenoma	0.0
94721_Donor 2 U -	0.0	72410_Kidney_HRCE	0.0

A_Mesenchymal Stem Cells			
94722_Donor 2 U - B_Mesenchymal Stem Cells	0.0	72411_Kidney_HRE	15.6
94723_Donor 2 U - C_Mesenchymal Stem Cells	0.6	73139_Uterus_Uterine smooth muscle cells	3.2

Table 29G. Panel 5D

Tissue Name	Rel. Exp.(%) Ag1749, Run 169269329	Tissue Name	Rel. Exp.(%) Ag1749, Run 169269329
97457_Patient-02go_adipose	1.6	94709_Donor 2 AM - A_adipose	0.0
97476_Patient-07sk_skeletal muscle	2.0	94710_Donor 2 AM - B_adipose	0.0
97477_Patient-07ut_uterus	0.0	94711_Donor 2 AM - C_adipose	0.0
97478_Patient-07pl_placenta	100.0	94712_Donor 2 AD - A_adipose	0.0
97481_Patient-08sk_skeletal muscle	3.5	94713_Donor 2 AD - B_adipose	0.0
97482_Patient-08ut_uterus	0.4	94714_Donor 2 AD - C_adipose	0.0
97483_Patient-08pl_placenta	14.1	94742_Donor 3 U - A_Mesenchymal Stem Cells	0.0
97486_Patient-09sk_skeletal muscle	0.6	94743_Donor 3 U - B_Mesenchymal Stem Cells	0.0
97487_Patient-09ut_uterus	0.0	94730_Donor 3 AM - A_adipose	0.0
97488_Patient-09pl_placenta	42.9	94731_Donor 3 AM - B_adipose	0.0
97492_Patient-10ut_uterus	0.0	94732_Donor 3 AM - C_adipose	0.2
97493_Patient-10pl_placenta	96.6	94733_Donor 3 AD - A_adipose	0.0
97495_Patient-11go_adipose	2.1	94734_Donor 3 AD - B_adipose	0.0
97496_Patient-11sk_skeletal muscle	0.7	94735_Donor 3 AD - C_adipose	0.0
97497_Patient-11ut_uterus	0.0	77138_Liver_HepG2untreated	0.3
97498_Patient-11pl_placenta	57.8	73556_Heart_Cardiac stromal cells (primary)	0.5
97500_Patient-12go_adipose	2.4	81735_Small Intestine	5.1
97501_Patient-12sk_skeletal muscle	2.9	72409_Kidney_Proximal Convoluted Tubule	0.0
97502_Patient-12ut_uterus	0.0	82685_Small intestine_Duodenum	0.0
97503_Patient-12pl_placenta	23.3	90650_Adrenal_Adrenocortical adenoma	0.0
94721_Donor 2 U - A_Mesenchymal Stem Cells	0.0	72410_Kidney_HRCE	0.0
94722_Donor 2 U - B_Mesenchymal Stem Cells	0.0	72411_Kidney_HRE	13.0
94723_Donor 2 U - C_Mesenchymal Stem Cells	0.0	73139_Uterus_Uterine smooth muscle cells	0.4

CNS_neurodegeneration_v1.0 Summary: Ag1749 This panel does not show differential expression of the CG56153-01 gene in Alzheimer's disease. However, this

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expression profile confirms the presence of this gene in the brain. Please see Panel 1.3D for discussion of this gene in the central nervous system.

Panel 1.3D Summary: Ag1749 The expression of the CG56153-01 gene is highest in a sample derived from the pituitary gland (CT=28.2). This is in concordance with published reports (see reference below). In addition, there is low but substantial expression in various brain tissues as well as placenta tissue. The expression pattern of this isoform of the neuronatin gene eludes to its developmental importance (see references below). Expression in this gene is higher in fetal lung (CT=33) and skeletal muscle (CT=30) than in the corresponding adult tissues. Thus, the expression of this gene could be used to distinguish pituitary gland tissue from other tissues in the panel. In addition, this gene and/or gene product can be used to differentiate between the adult and fetal forms of skeletal muscle and lung. Furthermore, the expression in fetal tissue suggests that this gene product may be involved in the development of these organs and thus may be useful in treating disease that affect the lung and skeletal muscle. This gene encodes a putative proteolipid that may function as a unique regulator of ion channels during brain development and therefore may also be useful in the treatment of neurodevelopmental disorders.

References:

Usui H, Morii K, Tanaka R, Tamura T, Washiyama K, Ichikawa T, Kumanishi T.cDNA cloning and mRNA expression analysis of the human neuronatin. High level expression in human pituitary gland and pituitary adenomas. J Mol Neurosci 1997 Aug;9(1):55-60

The authors cloned the nearly complete cDNA of human neuronatin with the aid of an expressed sequence tag (EST) database, and analyzed its expression in various human tissues by Northern blot analysis. The nucleotide and deduced amino acid sequences of the human neuronatin showed a high similarity to those of rodents. The Northern blot analysis revealed that the human neuronatin message was expressed predominantly in the fetal brain in the brain-specific manner, but only faintly in the adult brain. Among the various adult human tissues examined, the anterior pituitary gland was shown to be the only place where the neuronatin mRNA was strongly expressed. Intense neuronatin expression was also observed in several human pituitary adenomas, including ACTH-producing, GH-producing, and nonfunctioning adenomas, but hardly detected in other brain tumors.

PMID: 9356927

Dou D, Joseph R. Cloning of human neuronatin gene and its localization to chromosome-20q 11.2-12: the deduced protein is a novel "proteolipid'. Brain Res 1996 Jun 3;723(1-2):8-22

Human brain development is a continuum governed by differential gene expression.
5   Therefore, we proceeded to identify genes selectively expressed in the developing brain. Using differential display and library screening, a novel rat cDNA, neuronatin, was identified and used to screen a human fetal brain cDNA library. Human neuronatin cDNA was isolated and sequenced. The cDNA was 1159 bp long and corresponded in size to the 1.25 kb message detected on Northern analysis. Neuronatin mRNA was selectively expressed in human brain
10   during fetal development, but became repressed in adulthood. When studied in the rat, neuronatin mRNA first appeared at mid-gestation in association with the onset of neurogenesis, becoming most pronounced later in development when neuroepithelial proliferation and neuroblast commitment are manifest, and declined postnatally coinciding with the completion of neurogenesis. The deduced protein has two distinct domains, a
15   hydrophobic N-terminal and basic C-terminal rich in arginine residues. Both the amino acid sequence and secondary structure of this amphipathic polypeptide exhibited homology to PMP1 and phospholamban, members of the "proteolipid' class of proteins which function as regulatory subunits of membrane channels. The neuronatin gene, 3973 bases long, contains in its 5'-flanking region a neural restrictive silencer element which may govern neuron-specific
20   expression. Based on screening a somatic cell hybrid panel, neuronatin gene was assigned to chromosome-20. And, using deletion constructs of chromosome-20 and fluorescence in situ hybridization, neuronatin was localized to chromosome-20q11.2-12. In conclusion, neuronatin is a novel human gene that is developmentally regulated and expressed in the brain. The deduced protein is a proteolipid that may function as a unique regulator of ion channels during
25   brain development. The definitive localization of neuronatin to human chromosome 20q11.2-12 provides the basis to investigate this gene as a candidate in neuro-developmental diseases that may also map to this region.

PMID: 8813377

Panel 2D Summary: Ag1749 The expression of the CG56153-01 gene appears to be
30   highest in a sample derived from normal bladder tissue adjacent to a bladder malignancy (CT=33.6). In addition, there is substantial expression associated with normal stomach tissue, breast tissue and a number of normal colon tissue samples adjacent to malignant colon. This preferential expression in normal tissue samples is in agreement with the expression in Panel 1.3D. Thus, the expression of this gene could be used to distinguish this normal bladder tissue

sample from the other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be beneficial in the treatment of colon cancer, bladder cancer, breast cancer or gastric cancer.

Panel 4D Summary: Ag1749 The CG56153-01 gene, a neuronatin homolog is expressed at moderate to low levels in HUVEC cells resting, serum-starved, and activated with IFN-gamma, as well as resting astrocytes and kidney (CTs=32.8-33.44). This putative protein product is a proteolipid that may function as a regulator of ion channels in these cells and tissues, similar to its putative function in brain development. Antibodies and small molecules that antagonize the function of the CG56153-01 product may reduce or eliminate the symptoms in patients with autoimmune and inflammatory diseases in which endothelial cells and astrocytes are involved, such as lupus erythematosus, asthma, emphysema, Crohn's disease, ulcerative colitis, multiple sclerosis, rheumatoid arthritis, osteoarthritis, and psoriasis.

Panels 5 Islet and 5D Summary: Ag1749 The expression pattern of the CG56153-01 gene, which encodes a neuronatin isoform, indicates an importance in placental function and regulation. The placental tissue samples were collected from nondiabetic obese women (patients 7 and 9), a diabetic patient on insulin and classified as overweight (patient 10), and finally a women diagnosed with diabetes and on insulin (patient 12). Furthermore, the expression of this gene in placenta confirms the importance of the gene product in the development of the fetus.

SEC7 (CG56159-01)

Expression of gene CG56159-01 was assessed using the primer-probe sets Ag1910 and Ag2047, described in Tables 30A and 30B. Results of the RTQ-PCR runs are shown in Tables 30C, 30D and 30E.

Table 30A. Probe Name Ag1910

Primers	Sequences	Length	Start Position
Forward	5'-tcctgaacaggtacctgagcta-3' (SEQ ID NO:174)	22	2579
Probe	TET-5'-aagcaggacgccacctctaccatcat-3'-TAMRA (SEQ ID NO:175)	26	2626
Reverse	5'-caatgacgttgttggtaatgc-3' (SEQ ID NO:176)	21	2654

Table 30. Probe Name Ag2047

Primers	Sequences	Length	Start Position
Forward	5'-tcctgaacaggtacctgagcta-3' (SEQ ID NO:177)	22	2579
Probe	TET-5'-aagcaggacgccacctctaccatcat-3'-TAMRA (SEQ ID NO:178)	26	2626
Reverse	5'-caatgacgttgttggtaatgc-3' (SEQ ID NO:179)	21	2654

Table 30. Panel 1.3D

Tissue Name	Rel. Exp.(%)	Rel. Exp.(%)	Rel. Exp.(%)	Tissue Name	Rel. Exp.(%)	Rel. Exp.(%)	Rel. Exp.(%)

	Ag1910, Run 147571473	Ag1910, Run 165534505	Ag2047, Run 165627343		Ag1910, Run 147571473	Ag1910, Run 165534505	Ag2047, Run 165627343
Liver adenocarcinoma	0.0	0.1	0.1	Kidney (fetal)	5.6	7.9	10.3
Pancreas	27.7	51.1	73.7	Renal ca. 786-0	0.0	0.0	0.0
Pancreatic ca. CAPAN 2	0.0	0.0	0.0	Renal ca. A498	15.4	12.5	9.2
Adrenal gland	0.3	0.6	0.4	Renal ca. RXF 393	4.3	22.8	17.4
Thyroid	0.5	0.3	0.3	Renal ca. ACHN	0.0	0.0	0.0
Salivary gland	0.4	0.9	0.4	Renal ca. UO-31	1.3	1.3	1.4
Pituitary gland	0.2	0.2	0.3	Renal ca. TK-10	0.0	0.0	0.0
Brain (fetal)	0.0	0.4	0.1	Liver	6.0	25.0	20.9
Brain (whole)	0.2	1.8	0.3	Liver (fetal)	26.8	24.8	29.9
Brain (amygdala)	0.1	0.1	0.3	Liver ca. (hepatoblast) HepG2	11.4	10.5	8.3
Brain (cerebellum)	0.0	0.1	0.1	Lung	0.2	4.4	4.2
Brain (hippocampus)	0.1	0.4	0.5	Lung (fetal)	0.8	1.8	1.1
Brain (substantia nigra)	0.1	0.3	0.3	Lung ca. (small cell) LX-1	0.0	0.0	0.0
Brain (thalamus)	0.1	0.6	0.4	Lung ca. (small cell) NCI-H69	0.0	0.0	0.0
Cerebral Cortex	0.6	0.2	0.2	Lung ca. (s.cell var.) SHP-77	0.2	0.1	0.1
Spinal cord	0.9	1.5	1.0	Lung ca. (large cell)NCI-H460	0.0	0.1	0.4
glio/astro U87-MG	0.0	0.0	0.0	Lung ca. (non-sm. cell) A549	0.6	0.5	0.4
glio/astro U-118-MG	22.7	22.2	17.9	Lung ca. (non-s.cell) NCI-H23	0.3	0.3	0.3
astrocytoma SW1783	7.5	10.9	7.5	Lung ca. (non-s.cell) HOP-62	1.4	1.4	1.8
neuro*; met SK-N-AS	6.9	3.4	2.7	Lung ca. (non-s.cl) NCI-H522	0.0	0.0	0.0
astrocytoma SF-539	3.3	6.6	3.2	Lung ca. (squam.) SW 900	0.0	0.0	0.0
astrocytoma SNB-	5.3	4.5	3.7	Lung ca.	0.0	0.0	0.0

75				(squam.) NCI-H596			
glioma SNB-19	0.0	0.1	0.0	Mammary gland	7.7	6.3	2.7
glioma U251	0.0	0.1	0.0	Breast ca.* (pl.ef) MCF-7	0.0	0.1	0.0
glioma SF-295	0.0	0.1	0.0	Breast ca.* (pl.ef) MDA-MB-231	0.0	0.0	0.0
Heart (fetal)	2.0	0.3	0.2	Breast ca.* (pl.ef) T47D	0.0	0.0	0.0
Heart	0.1	0.6	0.6	Breast ca. BT-549	**100.0**	**100.0**	**100.0**
Skeletal muscle (fetal)	7.3	0.5	0.5	Breast ca. MDA-N	0.0	0.0	0.0
Skeletal muscle	0.1	0.7	0.5	Ovary	3.1	0.8	0.5
Bone marrow	4.2	5.6	5.8	Ovarian ca. OVCAR-3	0.2	0.1	0.1
Thymus	0.2	0.3	0.3	Ovarian ca. OVCAR-4	0.0	0.1	0.0
Spleen	1.0	2.1	1.7	Ovarian ca. OVCAR-5	0.0	0.0	0.0
Lymph node	0.5	1.8	1.2	Ovarian ca. OVCAR-8	1.3	1.9	1.0
Colorectal	3.3	1.6	1.3	Ovarian ca. IGROV-1	0.0	0.0	0.0
Stomach	2.7	2.4	1.9	Ovarian ca.* (ascites) SK-OV-3	8.2	11.3	10.4
Small intestine	27.2	94.0	73.7	Uterus	0.0	0.5	0.5
Colon ca. SW480	0.0	0.0	0.0	Placenta	1.5	1.1	1.1
Colon ca.* SW620(SW480 met)	0.0	0.0	0.0	Prostate	14.2	22.4	25.9
Colon ca. HT29	0.0	0.0	0.0	Prostate ca.* (bone met)PC-3	1.3	3.7	2.2
Colon ca. HCT-116	0.0	0.0	0.0	Testis	0.2	0.3	0.2
Colon ca. CaCo-2	1.0	0.2	0.2	Melanoma Hs688(A).T	28.3	4.3	4.2
Colon ca. tissue(ODO3866)	4.9	2.8	3.5	Melanoma* (met) Hs688(B).T	53.2	5.4	7.0
Colon ca. HCC-2998	0.0	0.0	0.0	Melanoma UACC-62	0.0	0.0	0.1
Gastric ca.* (liver met) NCI-N87	0.0	0.0	0.0	Melanoma M14	0.0	0.0	0.0
Bladder	14.8	22.5	19.6	Melanoma LOX IMVI	0.8	1.2	1.4
Trachea	1.2	2.3	2.0	Melanoma* (met) SK-MEL-5	0.0	0.0	0.0
Kidney	10.7	46.7	37.9	Adipose	1.8	1.0	0.9

Table30D. Panel 2.2

Tissue Name	Rel. Exp.(%) Ag1910, Run 174285074	Tissue Name	Rel. Exp.(%) Ag1910, Run 174285074
Normal Colon	6.5	Kidney Margin (OD04348)	24.1
Colon cancer (OD06064)	1.7	Kidney malignant cancer (OD06204B)	0.0
Colon Margin (OD06064)	20.4	Kidney normal adjacent tissue (OD06204E)	22.8
Colon cancer (OD06159)	0.2	Kidney Cancer (OD04450-01)	**100.0**
Colon Margin (OD06159)	28.3	Kidney Margin (OD04450-03)	10.7
Colon cancer (OD06297-04)	0.3	Kidney Cancer 8120613	0.1
Colon Margin (OD06297-015)	10.5	Kidney Margin 8120614	46.0
CC Gr.2 ascend colon (ODO3921)	2.4	Kidney Cancer 9010320	0.9
CC Margin (ODO3921)	4.7	Kidney Margin 9010321	17.6
Colon cancer metastasis (OD06104)	0.7	Kidney Cancer 8120607	0.6
Lung Margin (OD06104)	37.1	Kidney Margin 8120608	26.1
Colon mets to lung (OD04451-01)	0.2	Normal Uterus	0.1
Lung Margin (OD04451-02)	0.5	Uterine Cancer 064011	0.2
Normal Prostate	15.4	Normal Thyroid	0.2
Prostate Cancer (OD04410)	0.4	Thyroid Cancer 064010	0.1
Prostate Margin (OD04410)	2.1	Thyroid Cancer A302152	0.7
Normal Ovary	0.7	Thyroid Margin A302153	0.0
Ovarian cancer (OD06283-03)	0.5	Normal Breast	2.5
Ovarian Margin (OD06283-07)	0.3	Breast Cancer (OD04566)	1.6
Ovarian Cancer 064008	1.4	Breast Cancer 1024	1.8
Ovarian cancer (OD06145)	0.8	Breast Cancer (OD04590-01)	0.4
Ovarian Margin (OD06145)	0.7	Breast Cancer Mets (OD04590-03)	0.5
Ovarian cancer (OD06455-03)	0.0	Breast Cancer Metastasis (OD04655-05)	0.7
Ovarian Margin (OD06455-07)	0.2	Breast Cancer 064006	0.9
Normal Lung	0.9	Breast Cancer 9100266	0.6
Invasive poor diff. lung adeno (ODO4945-01	1.2	Breast Margin 9100265	0.7
Lung Margin (ODO4945-03)	2.1	Breast Cancer A209073	0.4
Lung Malignant Cancer (OD03126)	1.1	Breast Margin A2090734	2.8
Lung Margin (OD03126)	0.7	Breast cancer (OD06083)	1.8
Lung Cancer (OD05014A)	0.7	Breast cancer node metastasis (OD06083)	0.5
Lung Margin (OD05014B)	1.5	Normal Liver	22.7
Lung cancer (OD06081)	0.2	Liver Cancer 1026	10.4

Lung Margin (OD06081)	1.0	Liver Cancer 1025	40.3
Lung Cancer (OD04237-01)	0.3	Liver Cancer 6004-T	23.3
Lung Margin (OD04237-02)	2.0	Liver Tissue 6004-N	3.3
Ocular Melanoma Metastasis	0.0	Liver Cancer 6005-T	20.6
Ocular Melanoma Margin (Liver)	10.9	Liver Tissue 6005-N	41.5
Melanoma Metastasis	0.1	Liver Cancer 064003	4.8
Melanoma Margin (Lung)	1.7	Normal Bladder	13.9
Normal Kidney	9.6	Bladder Cancer 1023	0.5
Kidney Ca, Nuclear grade 2 (OD04338)	16.3	Bladder Cancer A302173	0.3
Kidney Margin (OD04338)	14.5	Normal Stomach	2.5
Kidney Ca Nuclear grade 1/2 (OD04339)	0.3	Gastric Cancer 9060397	2.2
Kidney Margin (OD04339)	32.1	Stomach Margin 9060396	7.3
Kidney Ca, Clear cell type (OD04340)	4.8	Gastric Cancer 9060395	0.8
Kidney Margin (OD04340)	9.3	Stomach Margin 9060394	24.5
Kidney Ca, Nuclear grade 3 (OD04348)	0.4	Gastric Cancer 064005	26.1

Table 30. Panel 4D

Tissue Name	Rel. Exp.(%) Ag1910, Run 159550646	Rel. Exp.(%) Ag2047, Run 161706228	Tissue Name	Rel. Exp.(%) Ag1910, Run 159550646	Rel. Exp.(%) Ag2047, Run 161706228
Secondary Th1 act	0.0	0.0	HUVEC IL-1beta	4.0	2.3
Secondary Th2 act	0.0	0.0	HUVEC IFN gamma	7.5	5.8
Secondary Tr1 act	0.0	0.1	HUVEC TNF alpha + IFN gamma	14.2	10.2
Secondary Th1 rest	0.0	0.0	HUVEC TNF alpha + IL4	19.9	17.7
Secondary Th2 rest	0.0	0.0	HUVEC IL-11	5.6	3.3
Secondary Tr1 rest	0.1	0.0	Lung Microvascular EC none	6.3	4.6
Primary Th1 act	0.0	0.0	Lung Microvascular EC TNFalpha + IL-1beta	4.3	4.1
Primary Th2 act	0.0	0.0	Microvascular Dermal EC none	6.3	5.6
Primary Tr1 act	0.0	0.0	Microsvasular Dermal EC TNFalpha + IL-1beta	4.2	2.9
Primary Th1 rest	0.1	0.0	Bronchial epithelium TNFalpha + IL1beta	0.3	0.4
Primary Th2 rest	0.1	0.0	Small airway epithelium none	0.4	0.3
Primary Tr1 rest	0.0	0.0	Small airway epithelium TNFalpha + IL-1beta	0.0	0.1
CD45RA CD4 lymphocyte act	5.2	2.7	Coronery artery SMC rest	6.1	4.4
CD45RO CD4 lymphocyte act	0.0	0.0	Coronery artery SMC TNFalpha + IL-1beta	4.9	2.9

CD8 lymphocyte act	0.0	0.0	Astrocytes rest	0.0	0.0
Secondary CD8 lymphocyte rest	0.0	0.0	Astrocytes TNFalpha + IL-1beta	0.1	0.1
Secondary CD8 lymphocyte act	0.0	0.0	KU-812 (Basophil) rest	1.2	0.8
CD4 lymphocyte none	0.0	0.0	KU-812 (Basophil) PMA/ionomycin	2.5	2.1
2ry Th1/Th2/Tr1_anti-CD95 CH11	0.1	0.1	CCD1106 (Keratinocytes) none	11.0	7.6
LAK cells rest	3.9	2.6	CCD1106 (Keratinocytes) TNFalpha + IL-1beta	1.2	1.2
LAK cells IL-2	0.0	0.0	Liver cirrhosis	2.8	1.7
LAK cells IL-2+IL-12	0.3	0.2	Lupus kidney	1.6	1.2
LAK cells IL-2+IFN gamma	0.7	0.1	NCI-H292 none	0.0	0.0
LAK cells IL-2+ IL-18	0.2	0.2	NCI-H292 IL-4	0.0	0.0
LAK cells PMA/ionomycin	16.3	12.8	NCI-H292 IL-9	0.0	0.0
NK Cells IL-2 rest	0.0	0.0	NCI-H292 IL-13	0.0	0.0
Two Way MLR 3 day	1.7	1.4	NCI-H292 IFN gamma	0.0	0.0
Two Way MLR 5 day	1.2	0.8	HPAEC none	5.9	4.2
Two Way MLR 7 day	0.2	0.2	HPAEC TNF alpha + IL-1 beta	9.0	5.8
PBMC rest	0.9	0.7	Lung fibroblast none	3.1	2.2
PBMC PWM	2.5	1.8	Lung fibroblast TNF alpha + IL-1 beta	6.0	5.0
PBMC PHA-L	1.8	1.6	Lung fibroblast IL-4	13.6	11.7
Ramos (B cell) none	0.0	0.0	Lung fibroblast IL-9	6.2	3.6
Ramos (B cell) ionomycin	0.0	0.0	Lung fibroblast IL-13	9.3	6.1
B lymphocytes PWM	0.1	0.1	Lung fibroblast IFN gamma	6.2	5.3
B lymphocytes CD40L and IL-4	0.3	0.3	Dermal fibroblast CCD1070 rest	19.3	15.6
EOL-1 dbcAMP	0.0	0.0	Dermal fibroblast CCD1070 TNF alpha	26.1	17.8
EOL-1 dbcAMP PMA/ionomycin	0.0	0.0	Dermal fibroblast CCD1070 IL-1 beta	18.3	12.8
Dendritic cells none	29.5	19.5	Dermal fibroblast IFN gamma	48.0	29.3
Dendritic cells LPS	29.5	24.0	Dermal fibroblast IL-4	88.3	62.4
Dendritic cells anti-CD40	23.0	13.9	IBD Colitis 2	0.0	0.0
Monocytes rest	5.6	3.3	IBD Crohn's	6.4	3.1
Monocytes LPS	20.7	16.5	Colon	**100.0**	**100.0**
Macrophages rest	8.1	6.5	Lung	2.0	1.4

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Macrophages LPS	13.4	8.7	Thymus	47.0	25.2
HUVEC none	19.6	17.2	Kidney	0.6	0.3
HUVEC starved	18.0	14.8			

Panel 1.3D Summary: Ag1910/Ag2047 Three experiments with the same probe and primer set produce results that are in excellent agreement, with highest expression of the CG56159-01 gene in a breast cancer cell line (BT549)(CTs=24-25). Thus, expression of this gene could be used to distinguish BT549 cells from other samples in the panel. There is also significant expression in clusters of cell lines derived from renal cancer, brain cancer and melanoma. This gene encodes a homolog of aminopeptidase N, which is thought to be critical in the metastasis of cancer by degrading extracellular matrix and aiding in cellular motility. Thus, therapeutic modulation of the expression or function of this protein may be useful in the treatment of these cancers or any metastatic cancer.

In addition, the expression of this homolog of Aminopeptidase N is moderate to high in several of the endocrine/metabolic tissues found on this panel, including adipose, liver, pancreas, skeletal muscle and small intestine. Aminopeptidase N (EC 3.4.11.2) is located in the small-intestinal and renal microvillar membranes, and also in other plasma membranes. In the small intestine, aminopeptidase N plays a role in the final digestion of peptides generated from hydrolysis of proteins by gastric and pancreatic proteases. Its function in proximal tubular epithelial cells and other cell types is less clear.

Panel 2.2 Summary: Ag1910 The expression of the CG56159-01 gene appears to be highest in a sample derived from a kidney cancer (CT=26.1). In addition, there appears to be substantial expression associated with liver derived tissue, normal colon tissue and a number of normal kidney tissue samples. This is consistent with the expression in Panel 1.3D and the function of this putative protein. (Please see Panel 1.3D for detailed discussion). Thus, the expression of this gene could be used to distinguish this kidney cancer sample from the other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, protein therapeutics or antibodies might be of benefit in the treatment of liver cancer, colon cancer or kidney cancer.

Panel 4D Summary: Ag1910/Ag2047 Two experiments with the same probe and primer set produce results that are in excellent agreement, with highest expression of the CG56159-01 gene in the colon (CTs=21-23). This is in concordance with the identification of this gene product as an aminopeptidase N homolog. Recently aminopeptidase N has been implicated in leukocyte chemotaxis and activation. Consistent with this presumed role in the

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immunologic events of inflammatory and allergic diseases, this gene product is also expressed in a wide range of other cell types of significance in the immune response in health and disease. Thus, significant levels of expression are also seen in treated and untreated lung and dermal fibroblasts and treated and untreated endothelial cells, macrophages and monocytes.

5   The transcript is more highly expressed in resting macrophages and monocytes than in treated cells of these types. Thus, the protein encoded by this transcript may be important in monocytic differentiation and activation and in conditions which involve endothelial cells. Therefore, regulating the expression of this transcript or the function of the protein it encodes could alter the types and levels of monocytic cells regulated by cytokine and chemokine

10   production and T cell activation. Furthermore, antibodies and small molecules that antagonize the function of the this product may reduce or eliminate the symptoms in patients with autoimmune and inflammatory diseases, such as lupus erythematosus, asthma, emphysema, Crohn's disease, ulcerative colitis, arthritis, and psoriasis.

       References:

15   Tani K, Ogushi F, Shimizu T, Sone S.Protease-induced leukocyte chemotaxis and activation: roles in host defense and inflammation. J Med Invest 2001 Aug;48(3-4):133-41

       The migration of leukocytes such as neutrophils, monocytes and lymphocytes into inflamed lesions is one of the critical events of inflammation. Although the traditional function of neutrophil-derived antimicrobial proteases is to ingest and kill bacteria, some neutrophil

20   serine proteases have been shown to induce leukocyte migration and activation. Mast cell-derived chymase also has the chemotactic activity for leukocytes. During the acute phase of inflammatory and allergic diseases, the predominantly migrated cells are neutrophils and mast cells, respectively, and in the subsequent chronic phase, monocytes and lymphocytes are mainly migrated. The chemotactic activity for monocytes and lymphocytes of neutrophil-

25   derived serine proteases and mast cell-derived chymase may have a role in switching acute inflammation to chronic inflammation and delayed-type hypersensitivity. Recently, aminopeptidase N and endothelin were shown to induce chemotactic migration of leukocytes. Thus, protease-induced leukocyte chemotaxis and activation may play an important role in immunologic events of inflammatory and allergic diseases.

30   PMID: 11694952

       SEC9 (CG56162-01)

Expression of gene CG56162-01 was assessed using the primer-probe sets Ag1952, Ag1906 and Ag2042, described in Tables 31A, 31B and 31C. Results of the RTQ-PCR runs are shown in Tables 31D, 31E, 31F, 31G and 31H.

Table 31A. Probe Name Ag1952

Primers	Sequences	Length	Start Position
Forward	5'-gaccagggtcatatttgcacta-3' (SEQ ID NO:180)	22	1916
Probe	TET-5'-cctcctgaggtaacagcaagtcccat-3'-TAMRA (SEQ ID NO:181)	26	1943
Reverse	5'-cgggaatactttccccttcta-3' (SEQ ID NO:182)	21	1970

Table 31B. Probe Name Ag1906

Primers	Sequences	Length	Start Position
Forward	5'-gtactcatttcgcctctggtt-3' (SEQ ID NO:183)	21	3895
Probe	TET-5'-tgcaacaactttcaaggtccttgctg-3'-TAMRA (SEQ ID NO:184)	26	3852
Reverse	5'-agcacaaggttgagcactttc-3' (SEQ ID NO:185)	21	3830

Table 31C. Probe Name Ag2042

Primers	Sequences	Length	Start Position
Forward	5'-cataatggaaacaggacctgaa-3' (SEQ ID NO:186)	22	4354
Probe	TET-5'-ccttccagcatgccagaggaaagtt-3'-TAMRA (SEQ ID NO:187)	25	4326
Reverse	5'-aggtcctggtagggaatgct-3' (SEQ ID NO:188)	20	4286

Table 31D. CNS_neurodegeneration_v1.0

Tissue Name	Rel. Exp.(%) Ag1952, Run 207776409	Tissue Name	Rel. Exp.(%) Ag1952, Run 207776409
AD 1 Hippo	18.0	Control (Path) 3 Temporal Ctx	22.7
AD 2 Hippo	43.8	Control (Path) 4 Temporal Ctx	46.7
AD 3 Hippo	12.1	AD 1 Occipital Ctx	22.1
AD 4 Hippo	9.0	AD 2 Occipital Ctx (Missing)	0.0
AD 5 Hippo	**100.0**	AD 3 Occipital Ctx	20.6
AD 6 Hippo	46.7	AD 4 Occipital Ctx	35.8
Control 2 Hippo	24.7	AD 5 Occipital Ctx	49.7
Control 4 Hippo	16.7	AD 6 Occipital Ctx	23.0
Control (Path) 3 Hippo	11.8	Control 1 Occipital Ctx	17.7
AD 1 Temporal Ctx	26.6	Control 2 Occipital Ctx	71.7
AD 2 Temporal Ctx	51.8	Control 3 Occipital Ctx	27.7
AD 3 Temporal Ctx	24.0	Control 4 Occipital Ctx	17.8
AD 4 Temporal Ctx	37.9	Control (Path) 1 Occipital Ctx	95.9
AD 5 Inf Temporal Ctx	93.3	Control (Path) 2 Occipital Ctx	17.8
AD 5 Sup Temporal Ctx	44.8	Control (Path) 3 Occipital Ctx	12.6
AD 6 Inf Temporal Ctx	59.5	Control (Path) 4 Occipital Ctx	27.4
AD 6 Sup Temporal Ctx	59.9	Control 1 Parietal Ctx	22.5
Control 1 Temporal Ctx	28.1	Control 2 Parietal Ctx	64.2

Control 2 Temporal Ctx	68.8	Control 3 Parietal Ctx	23.2
Control 3 Temporal Ctx	31.0	Control (Path) 1 Parietal Ctx	98.6
Control 3 Temporal Ctx	21.0	Control (Path) 2 Parietal Ctx	37.1
Control (Path) 1 Temporal Ctx	94.6	Control (Path) 3 Parietal Ctx	17.6
Control (Path) 2 Temporal Ctx	61.1	Control (Path) 4 Parietal Ctx	64.2

Table 31E. Panel 1.3D

Tissue Name	Rel. Exp.(%) Ag1906, Run 147697143	Rel. Exp.(%) Ag2042, Run 165627321	Tissue Name	Rel. Exp.(%) Ag1906, Run 147697143	Rel. Exp.(%) Ag2042, Run 165627321
Liver adenocarcinoma	4.5	5.8	Kidney (fetal)	9.0	6.4
Pancreas	0.7	0.8	Renal ca. 786-0	17.9	25.7
Pancreatic ca. CAPAN 2	3.2	8.5	Renal ca. A498	53.6	27.0
Adrenal gland	3.2	1.6	Renal ca. RXF 393	9.5	46.0
Thyroid	3.9	1.4	Renal ca. ACHN	23.0	14.7
Salivary gland	3.3	6.2	Renal ca. UO-31	24.1	41.8
Pituitary gland	3.0	1.6	Renal ca. TK-10	10.8	8.5
Brain (fetal)	6.7	33.7	Liver	3.8	3.5
Brain (whole)	26.4	92.0	Liver (fetal)	4.5	0.9
Brain (amygdala)	17.7	37.9	Liver ca. (hepatoblast) HepG2	0.0	0.0
Brain (cerebellum)	9.8	94.6	Lung	13.3	8.8
Brain (hippocampus)	27.2	50.7	Lung (fetal)	9.6	12.1
Brain (substantia nigra)	5.0	11.6	Lung ca. (small cell) LX-1	1.0	2.1
Brain (thalamus)	16.6	51.1	Lung ca. (small cell) NCI-H69	5.8	6.1
Cerebral Cortex	**100.0**	76.3	Lung ca. (s.cell var.) SHP-77	0.2	0.0
Spinal cord	13.4	12.0	Lung ca. (large cell)NCI-H460	0.2	1.6
glio/astro U87-MG	39.2	28.3	Lung ca. (non-sm. cell) A549	10.7	6.4
glio/astro U-118-MG	54.7	**100.0**	Lung ca. (non-s.cell) NCI-H23	0.1	0.4
astrocytoma SW1783	2.9	3.2	Lung ca. (non-s.cell) HOP-62	0.4	0.0
neuro*; met SK-N-AS	0.5	0.3	Lung ca. (non-s.cl) NCI-H522	0.0	0.0
astrocytoma SF-539	17.9	40.9	Lung ca. (squam.) SW 900	8.9	12.6
astrocytoma SNB-75	20.9	12.3	Lung ca. (squam.) NCI-H596	3.3	9.0
glioma SNB-19	10.4	4.1	Mammary gland	49.3	9.5
glioma U251	1.5	1.5	Breast ca.*	5.1	5.8

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			(pl.ef) MCF-7		
glioma SF-295	5.3	1.4	Breast ca.* (pl.ef) MDA-MB-231	11.0	21.9
Heart (fetal)	34.6	6.0	Breast ca.* (pl.ef) T47D	0.6	0.4
Heart	12.9	12.8	Breast ca. BT-549	0.2	0.3
Skeletal muscle (fetal)	88.9	8.5	Breast ca. MDA-N	4.7	1.3
Skeletal muscle	6.6	6.2	Ovary	25.5	2.1
Bone marrow	4.3	2.3	Ovarian ca. OVCAR-3	0.5	0.0
Thymus	3.0	1.0	Ovarian ca. OVCAR-4	3.2	7.6
Spleen	13.0	13.3	Ovarian ca. OVCAR-5	12.1	10.2
Lymph node	6.1	5.7	Ovarian ca. OVCAR-8	9.0	10.6
Colorectal	18.4	10.5	Ovarian ca. IGROV-1	3.8	3.2
Stomach	9.4	5.5	Ovarian ca.* (ascites) SK-OV-3	35.8	45.7
Small intestine	7.7	10.2	Uterus	5.0	8.7
Colon ca. SW480	1.3	0.3	Placenta	17.0	4.3
Colon ca.* SW620(SW480 met)	0.0	0.0	Prostate	1.7	1.3
Colon ca. HT29	10.4	8.1	Prostate ca.* (bone met)PC-3	18.6	22.2
Colon ca. HCT-116	0.8	2.4	Testis	3.6	0.8
Colon ca. CaCo-2	4.5	0.8	Melanoma Hs688(A).T	24.7	1.4
Colon ca. tissue(ODO3866)	13.2	10.8	Melanoma* (met) Hs688(B).T	48.6	4.5
Colon ca. HCC-2998	4.5	2.2	Melanoma UACC-62	2.3	3.0
Gastric ca.* (liver met) NCI-N87	6.6	7.3	Melanoma M14	0.0	0.0
Bladder	0.8	1.5	Melanoma LOX IMVI	0.5	1.4
Trachea	17.6	11.6	Melanoma* (met) SK-MEL-5	0.3	0.1
Kidney	5.3	5.1	Adipose	20.2	9.2

Table 31F Panel 2.2

Tissue Name	Rel. Exp.(%) Ag1906, Run 174153439	Tissue Name	Rel. Exp.(%) Ag1906, Run 174153439
Normal Colon	19.6	Kidney Margin (OD04348)	**100.0**
Colon cancer (OD06064)	18.2	Kidney malignant cancer (OD06204B)	1.2
Colon Margin (OD06064)	25.9	Kidney normal adjacent tissue (OD06204E)	28.9

Colon cancer (OD06159)	7.7	Kidney Cancer (OD04450-01)	25.3
Colon Margin (OD06159)	25.0	Kidney Margin (OD04450-03)	20.0
Colon cancer (OD06297-04)	5.9	Kidney Cancer 8120613	5.1
Colon Margin (OD06297-015)	41.5	Kidney Margin 8120614	36.1
CC Gr.2 ascend colon (ODO3921)	6.6	Kidney Cancer 9010320	16.4
CC Margin (ODO3921)	12.9	Kidney Margin 9010321	22.4
Colon cancer metastasis (OD06104)	8.2	Kidney Cancer 8120607	98.6
Lung Margin (OD06104)	11.2	Kidney Margin 8120608	23.2
Colon mets to lung (OD04451-01)	35.8	Normal Uterus	14.6
Lung Margin (OD04451-02)	38.2	Uterine Cancer 064011	5.5
Normal Prostate	3.0	Normal Thyroid	2.7
Prostate Cancer (OD04410)	1.3	Thyroid Cancer 064010	1.6
Prostate Margin (OD04410)	4.6	Thyroid Cancer A302152	8.1
Normal Ovary	11.2	Thyroid Margin A302153	1.6
Ovarian cancer (OD06283-03)	1.9	Normal Breast	26.4
Ovarian Margin (OD06283-07)	22.4	Breast Cancer (OD04566)	4.1
Ovarian Cancer 064008	5.1	Breast Cancer 1024	20.7
Ovarian cancer (OD06145)	6.4	Breast Cancer (OD04590-01)	51.1
Ovarian Margin (OD06145)	15.0	Breast Cancer Mets (OD04590-03)	25.2
Ovarian cancer (OD06455-03)	5.9	Breast Cancer Metastasis (OD04655-05)	9.1
Ovarian Margin (OD06455-07)	7.1	Breast Cancer 064006	6.4
Normal Lung	21.2	Breast Cancer 9100266	13.6
Invasive poor diff. lung adeno (ODO4945-01	5.0	Breast Margin 9100265	8.4
Lung Margin (ODO4945-03)	16.0	Breast Cancer A209073	4.9
Lung Malignant Cancer (OD03126)	13.1	Breast Margin A2090734	11.3
Lung Margin (OD03126)	12.7	Breast cancer (OD06083)	22.7
Lung Cancer (OD05014A)	17.0	Breast cancer node metastasis (OD06083)	19.1
Lung Margin (OD05014B)	40.1	Normal Liver	42.6
Lung cancer (OD06081)	3.8	Liver Cancer 1026	17.4
Lung Margin (OD06081)	16.8	Liver Cancer 1025	59.0
Lung Cancer (OD04237-01)	2.7	Liver Cancer 6004-T	55.1
Lung Margin (OD04237-02)	52.1	Liver Tissue 6004-N	3.1
Ocular Melanoma Metastasis	0.4	Liver Cancer 6005-T	43.8
Ocular Melanoma Margin (Liver)	32.5	Liver Tissue 6005-N	83.5
Melanoma Metastasis	1.4	Liver Cancer 064003	17.3
Melanoma Margin (Lung)	28.9	Normal Bladder	3.4

Normal Kidney	13.2	Bladder Cancer 1023	11.6
Kidney Ca, Nuclear grade 2 (OD04338)	45.4	Bladder Cancer A302173	2.9
Kidney Margin (OD04338)	2.8	Normal Stomach	34.9
Kidney Ca Nuclear grade 1/2 (OD04339)	76.8	Gastric Cancer 9060397	9.2
Kidney Margin (OD04339)	25.5	Stomach Margin 9060396	16.6
Kidney Ca, Clear cell type (OD04340)	47.6	Gastric Cancer 9060395	21.8
Kidney Margin (OD04340)	23.3	Stomach Margin 9060394	37.4
Kidney Ca, Nuclear grade 3 (OD04348)	4.4	Gastric Cancer 064005	10.7

Table 31G. Panel 2D

Tissue Name	Rel. Exp.(%) Ag1952, Run 162734662	Tissue Name	Rel. Exp.(%) Ag1952, Run 162734662
Normal Colon	72.2	Kidney Margin 8120608	30.4
CC Well to Mod Diff (ODO3866)	13.3	Kidney Cancer 8120613	11.8
CC Margin (ODO3866)	34.9	Kidney Margin 8120614	30.4
CC Gr.2 rectosigmoid (ODO3868)	8.0	Kidney Cancer 9010320	43.2
CC Margin (ODO3868)	5.7	Kidney Margin 9010321	55.1
CC Mod Diff (ODO3920)	5.4	Normal Uterus	4.0
CC Margin (ODO3920)	22.8	Uterus Cancer 064011	10.1
CC Gr.2 ascend colon (ODO3921)	35.1	Normal Thyroid	9.3
CC Margin (ODO3921)	38.2	Thyroid Cancer 064010	3.3
CC from Partial Hepatectomy (ODO4309) Mets	49.7	Thyroid Cancer A302152	5.3
Liver Margin (ODO4309)	34.9	Thyroid Margin A302153	4.1
Colon mets to lung (OD04451-01)	6.7	Normal Breast	25.3
Lung Margin (OD04451-02)	34.9	Breast Cancer (OD04566)	4.7
Normal Prostate 6546-1	60.7	Breast Cancer (OD04590-01)	46.0
Prostate Cancer (OD04410)	6.2	Breast Cancer Mets (OD04590-03)	66.9
Prostate Margin (OD04410)	7.4	Breast Cancer Metastasis (OD04655-05)	5.8
Prostate Cancer (OD04720-01)	6.5	Breast Cancer 064006	5.3
Prostate Margin (OD04720-02)	19.2	Breast Cancer 1024	20.3
Normal Lung 061010	64.6	Breast Cancer 9100266	40.1
Lung Met to Muscle (ODO4286)	13.6	Breast Margin 9100265	12.9
Muscle Margin (ODO4286)	22.4	Breast Cancer A209073	28.9
Lung Malignant Cancer (OD03126)	33.0	Breast Margin A2090734	6.7
Lung Margin (OD03126)	45.7	Normal Liver	26.2
Lung Cancer (OD04404)	11.4	Liver Cancer 064003	13.9
Lung Margin (OD04404)	23.8	Liver Cancer 1025	31.4
Lung Cancer (OD04565)	1.9	Liver Cancer 1026	20.4

Lung Margin (OD04565)	14.7	Liver Cancer 6004-T	42.3
Lung Cancer (OD04237-01)	5.2	Liver Tissue 6004-N	2.5
Lung Margin (OD04237-02)	40.6	Liver Cancer 6005-T	18.8
Ocular Mel Met to Liver (ODO4310)	1.2	Liver Tissue 6005-N	7.7
Liver Margin (ODO4310)	24.5	Normal Bladder	7.6
Melanoma Mets to Lung (OD04321)	2.1	Bladder Cancer 1023	9.7
Lung Margin (OD04321)	57.8	Bladder Cancer A302173	2.3
Normal Kidney	50.3	Bladder Cancer (OD04718-01)	17.3
Kidney Ca, Nuclear grade 2 (OD04338)	13.1	Bladder Normal Adjacent (OD04718-03)	17.4
Kidney Margin (OD04338)	31.0	Normal Ovary	4.2
Kidney Ca Nuclear grade 1/2 (OD04339)	29.5	Ovarian Cancer 064008	6.9
Kidney Margin (OD04339)	48.0	Ovarian Cancer (OD04768-07)	64.2
Kidney Ca, Clear cell type (OD04340)	**100.0**	Ovary Margin (OD04768-08)	5.0
Kidney Margin (OD04340)	31.4	Normal Stomach	26.8
Kidney Ca, Nuclear grade 3 (OD04348)	8.0	Gastric Cancer 9060358	7.4
Kidney Margin (OD04348)	27.0	Stomach Margin 9060359	12.9
Kidney Cancer (OD04622-01)	71.2	Gastric Cancer 9060395	36.6
Kidney Margin (OD04622-03)	10.7	Stomach Margin 9060394	28.5
Kidney Cancer (OD04450-01)	8.2	Gastric Cancer 9060397	42.0
Kidney Margin (OD04450-03)	25.2	Stomach Margin 9060396	10.7
Kidney Cancer 8120607	74.7	Gastric Cancer 064005	35.1

Table 31H. Panel 4D

Tissue Name	Rel. Exp.(%) Ag1906, Run 160658067	Rel. Exp.(%) Ag1952, Run 163578848	Rel. Exp.(%) Ag1952, Run 163593578	Rel. Exp.(%) Ag2042, Run 161383006
Secondary Th1 act	0.3	0.2	0.2	0.3
Secondary Th2 act	1.1	1.8	1.1	0.7
Secondary Tr1 act	1.5	0.8	1.1	1.0
Secondary Th1 rest	0.0	0.1	0.2	0.0
Secondary Th2 rest	2.5	3.6	2.4	1.5
Secondary Tr1 rest	1.1	3.3	2.3	0.9
Primary Th1 act	0.7	0.7	0.5	0.9
Primary Th2 act	1.4	1.3	1.0	1.9
Primary Tr1 act	0.9	0.7	0.8	0.4
Primary Th1 rest	0.9	1.0	1.0	1.2
Primary Th2 rest	1.3	1.8	2.4	1.5
Primary Tr1 rest	0.6	0.4	0.8	0.8
CD45RA CD4 lymphocyte act	10.2	12.9	18.0	15.3
CD45RO CD4 lymphocyte act	0.7	0.6	0.4	0.4
CD8 lymphocyte act	0.8	0.5	0.4	1.2
Secondary CD8 lymphocyte	0.5	1.0	0.6	0.2

rest				
Secondary CD8 lymphocyte act	2.6	3.7	3.2	3.0
CD4 lymphocyte none	0.1	0.2	0.3	0.1
2ry Th1/Th2/Tr1_anti-CD95 CH11	1.3	1.5	1.7	1.3
LAK cells rest	10.0	9.3	9.5	3.3
LAK cells IL-2	0.2	0.3	0.2	0.2
LAK cells IL-2+IL-12	1.6	1.8	1.7	1.2
LAK cells IL-2+IFN gamma	2.8	3.8	2.8	3.2
LAK cells IL-2+ IL-18	2.5	1.7	3.5	1.7
LAK cells PMA/ionomycin	4.8	3.3	2.9	2.1
NK Cells IL-2 rest	0.1	0.2	0.3	0.0
Two Way MLR 3 day	10.6	15.5	13.0	7.3
Two Way MLR 5 day	6.0	8.3	7.7	2.5
Two Way MLR 7 day	1.9	2.7	1.7	1.3
PBMC rest	0.8	0.5	0.7	0.4
PBMC PWM	9.9	11.8	12.8	9.2
PBMC PHA-L	4.5	3.9	4.1	2.4
Ramos (B cell) none	0.1	0.0	0.0	0.0
Ramos (B cell) ionomycin	0.2	0.0	0.1	0.0
B lymphocytes PWM	10.7	10.7	11.7	10.7
B lymphocytes CD40L and IL-4	10.2	10.8	10.0	8.3
EOL-1 dbcAMP	0.1	0.2	0.3	0.0
EOL-1 dbcAMP PMA/ionomycin	1.2	0.8	1.0	0.1
Dendritic cells none	19.5	21.8	23.3	7.3
Dendritic cells LPS	68.3	75.3	66.0	28.1
Dendritic cells anti-CD40	22.8	25.3	23.5	13.0
Monocytes rest	1.2	1.0	1.0	0.1
Monocytes LPS	19.8	19.9	22.7	16.5
Macrophages rest	**100.0**	91.4	**100.0**	51.4
Macrophages LPS	32.1	30.6	25.2	18.0
HUVEC none	26.6	22.8	31.2	45.1
HUVEC starved	25.9	25.7	33.0	54.7
HUVEC IL-1beta	14.9	14.1	9.9	22.8
HUVEC IFN gamma	10.4	11.3	11.3	17.4
HUVEC TNF alpha + IFN gamma	40.3	51.4	46.7	59.0
HUVEC TNF alpha + IL4	35.8	47.0	38.7	54.0
HUVEC IL-11	6.0	6.4	6.4	14.1
Lung Microvascular EC none	22.5	33.0	25.5	42.0
Lung Microvascular EC TNFalpha + IL-1beta	48.6	70.7	52.5	79.6
Microvascular Dermal EC none	36.3	45.1	33.7	54.3
Microvasular Dermal EC TNFalpha + IL-1beta	46.7	79.0	54.7	54.3

Bronchial epithelium TNFalpha + IL1beta	4.8	48.0	3.9	7.8
Small airway epithelium none	10.1	14.2	7.9	12.8
Small airway epithelium TNFalpha + IL-1beta	33.7	40.6	42.6	41.5
Coronery artery SMC rest	6.5	8.1	7.1	0.0
Coronery artery SMC TNFalpha + IL-1beta	5.4	7.3	9.0	7.3
Astrocytes rest	1.1	1.4	0.6	0.7
Astrocytes TNFalpha + IL-1beta	5.6	7.6	6.1	3.6
KU-812 (Basophil) rest	0.1	0.2	0.3	0.0
KU-812 (Basophil) PMA/ionomycin	0.2	0.2	0.6	0.3
CCD1106 (Keratinocytes) none	12.1	12.6	10.1	18.0
CCD1106 (Keratinocytes) TNFalpha + IL-1beta	0.7	9.0	1.6	1.0
Liver cirrhosis	2.8	5.6	3.5	3.5
Lupus kidney	3.2	7.0	7.6	5.4
NCI-H292 none	9.4	10.1	11.7	16.3
NCI-H292 IL-4	10.0	11.8	14.3	24.1
NCI-H292 IL-9	11.0	16.5	16.0	23.3
NCI-H292 IL-13	7.5	12.5	8.7	11.9
NCI-H292 IFN gamma	7.9	15.0	9.6	21.9
HPAEC none	16.2	21.0	17.0	25.7
HPAEC TNF alpha + IL-1 beta	54.3	**100.0**	60.3	64.6
Lung fibroblast none	13.9	23.0	19.8	18.8
Lung fibroblast TNF alpha + IL-1 beta	23.8	36.9	46.7	33.7
Lung fibroblast IL-4	27.7	43.5	32.5	29.5
Lung fibroblast IL-9	26.8	38.4	34.4	43.2
Lung fibroblast IL-13	15.3	19.3	19.9	18.7
Lung fibroblast IFN gamma	28.5	34.4	29.9	29.1
Dermal fibroblast CCD1070 rest	47.6	69.3	55.9	64.6
Dermal fibroblast CCD1070 TNF alpha	53.6	60.3	69.7	**100.0**
Dermal fibroblast CCD1070 IL-1 beta	43.8	63.3	58.6	48.0
Dermal fibroblast IFN gamma	23.5	43.5	36.3	24.0
Dermal fibroblast IL-4	39.0	58.6	59.0	41.5
IBD Colitis 2	0.2	0.5	0.2	0.4
IBD Crohn's	1.4	1.8	1.2	1.0
Colon	14.9	32.1	29.5	18.4
Lung	38.7	55.5	59.0	44.1
Thymus	23.0	37.6	28.3	17.2
Kidney	4.0	4.8	5.3	5.4

CNS_neurodegeneration_v1.0 Summary: Ag1952 This panel does not show differential expression of the CG56162-01 gene in Alzheimer's disease. However, this expression profile confirms the presence of this gene in the brain. Please see Panel 1.3D for discussion of this gene in the central nervous system.

Panel 1.3D Summary: Ag1906/Ag2042 The expression of CG56162-01 gene was assessed in two independent runs using 2 different probe/primer pairs, with good concordance between the runs. Highest expression in this panel is seen in brain-derived tissue, including the cerebral cortex and a brain cancer (CTs=25-27). Thus, the expression of this gene could be used to distinguish these samples from other samples in the panel.

This gene encodes a lysophosphase homolog that also has high levels of expression in many of the endocrine/metabolic tissues found on this panel, including adipose, liver, pancreas, pituitary, skeletal muscle and small intestine. Lysophospholipids are detergent-like intermediates in phospholipid metabolism. Lysophospholipases are important enzymes in the regulation of hormone biosynthesis and metabolism, and have been shown to be important in the regulation of insulin secretion (see reference below). Increased lysophospholipids levels have been detected in a variety of diseases including atherosclerosis and hyperlipidemia. In some cases, increased levels of lysophospholipids are hypothesized to result from a dysfunction of lysophospholipids-regulating enzymes including lysophospholipases, which act on biologic membranes to regulate the level of lysophospholipids by hydrolysis. Thus, this gene product may be useful in the treatment of diseases associated with increased lysophospholipids.

This gene also shows high expression in the brain. Lysophospholipases are critical enzymes that regulate brain membrane phospholipids. Alterations in their activity have been associated with a host of neurological disorders, including schizophrenia, Parkinson's disease, and Alzheimer's disease. Thus, therapeutic modulation of the expression or function of this gene or gene product may be useful in the treatment of these diseases. Please note that results from a third experiment with the probe/primer set Ag1952 are not included. The amp plot indicates that there were experimental difficulties with this run.

References:

Capito K, Reinsmark R, Thams P. Mechanism of fat-induced attenuation of glucose-induced insulin secretion from mouse pancreatic islets. Acta Diabetol 1999 Dec;36(3):119-25

In order to investigate the mechanism behind fat-induced inhibition of glucose-induced insulin secretion a selection of enzymes that may participate in regulation of pancreatic islet glucose oxidation was studied in islets isolated from mice that had been fed on a laboratory chow diet or on a high-fat diet for 10-12 weeks. At 20 mmol/L glucose production of

5   (14)CO(2) from [U-(14)C]-glucose was decreased 50% in islets from fat-fed mice. At 3.3 mmol/L glucose the glucose oxidation rate was similar in the two groups. The fatinduced decrease in glucose oxidation rate was correlated with a 35% decrease in the maximal glucokinase activity. The K(m) for glucose was unchanged. No differences between the diet groups were found in the activities of hexokinase, phosphofructo-1-kinase, glucose 6-

10   phosphatase or mitochondrial glycerophosphate dehydrogenase. After preincubation with 20 mmol/L glucose the activity of cytosolic Ca(2+)-independent as well as Ca(2+)-dependent phospholipase A(2) was unchanged by fat-feeding. However, the activity of lysophospholipase was significantly increased by fat feeding, which may result in lowered concentrations of islet lysophosphatidylcholine (lysoPC). It is concluded that in fat-induced diabetic animals a

15   decrease in islet glucokinase may contribute considerably to the decrease in islet glucose oxidation rate. Furthermore, the study raises the possibility that changes in islet lysoPC may contribute to the fat-induced attenuation of glucose-induced insulin secretion.

PMID: 10664315

Ross BM, Turenne S, Moszczynska A, Warsh JJ, Kish SJ.Differential alteration of
20   phospholipase A2 activities in brain of patients with schizophrenia. Brain Res 1999 Mar 13;821(2):407-13

We recently reported that the activity of a calcium-independent subtype of phospholipase A2 is increased in blood of patients with schizophrenia. The present investigation examined whether similar changes take place in brain of patients with this

25   disorder, and for comparison, in patients with bipolar disorder. The activity of two classes of PLA2, calcium-stimulated and independent, were assayed in autopsied temporal, prefrontal and occipital cortices, putamen, hippocampus and thalamus of 10 patients with schizophrenia, 8 patients with bipolar disorder and 12 matched control subjects. Calcium-independent PLA2 activity was increased by 45% in the temporal cortex of patients with schizophrenia as

30   compared with the controls but was not significantly altered in other brain areas. In contrast, calcium-stimulated PLA2 activity was decreased by 27-42% in the temporal and prefrontal cortices and putamen, with no significant alterations in other brain regions. Brain PLA2 activity was normal in patients with bipolar disorder. Calcium-stimulated PLA2 activity was normal in cortex, cerebellum and striatum of rats treated acutely or chronically with

haloperidol, whereas calcium-independent PLA2 activity was decreased in striatum of chronically treated animals, indicating that altered PLA2 activity in patients with schizophrenia is unlikely to be a direct effect of medication. Studies of the cellular role played by PLA2 suggest that decreased calcium-stimulated PLA2 activity, as also occurs in striatum

5   of chronic human cocaine users, may be due, in part, to increased dopaminergic activity in the disorder, whereas increased calcium-independent PLA2 activity may be related to abnormal fatty acid metabolism and oxidative stress in schizophrenia. Copyright 1999 Elsevier Science B.V.

Ross BM, Moszczynska A, Erlich J, Kish SJ. Low activity of key phospholipid

10   catabolic and anabolic enzymes in human substantia nigra: possible implications for Parkinson's disease. Neuroscience 1998 Apr;83(3):791-8

To determine whether increased oxidative stress in substantia nigra of patients with idiopathic Parkinson's disease might be related to decreased ability of nigral cells to detoxify oxidized membrane phospholipids, we compared levels of the major phospholipid

15   metabolizing enzymes in autopsied substantia nigra with those in non-nigral (n = 11) brain areas of the normal human brain. Whereas most enzymes possessed a relatively homogeneous distribution, the activity of the major phospholipid catabolizing enzyme phospholipase A2, assayed in the presence of calcium ions, varied amongst different regions, with substantia nigra possessing the lowest activity. Similarly, calcium-independent phospholipase A2

20   activity, although possessing a relatively homogeneous regional distribution, was also low in the substantia nigra. This, coupled with low activity of phosphoethanolamine- and phosphocholine-cytidylyltransferases, major regulatory enzymes of phospholipid synthesis, in this brain region, suggest that the rate of phospholipid turnover is low in the substantia nigra. Low activity of key phospholipid catabolic and anabolic enzymes in human substantia nigra

25   might result in reduced ability to repair oxidative membrane damage, as may occur in Parkinson's disease.

PMID: 9483562

Ross BM, Moszczynska A, Erlich J, Kish SJ.Phospholipid-metabolizing enzymes in Alzheimer's disease: increased lysophospholipid acyltransferase activity and decreased

30   phospholipase A2 activity. J Neurochem 1998 Feb;70(2):786-93

Damage to brain membrane phospholipids may play an important role in the pathogenesis of Alzheimer's disease (AD); however, the critical metabolic processes responsible for the generation and repair of membrane phospholipids affected by the disease

are unknown. We measured the activity of key phospholipid catabolic and anabolic enzymes in morphologically affected and spared areas of autopsied brain of patients with AD and in matched control subjects. The activity of the major catabolic enzyme phospholipase A2 (PLA2), measured in both the presence and absence of Ca2+, was significantly decreased (-35 to -53%) in parietal and temporal cortices of patients with AD. In contrast, the activities of lysophospholipid acyltransferase, which recycles lysophospholipids into intact phospholipids, and glycerophosphocholine phosphodiesterase, which returns phospholipid catabolites to be used in phospholipid resynthesis, were increased by approximately 50-70% in the same brain areas. Brain activities of enzymes involved in de novo phospholipid synthesis (ethanolamine kinase, choline kinase, choline phosphotransferase, phosphoethanolamine cytidylyltransferase, and phosphocholine cytidylyltransferase) were either normal or only slightly altered. The activities of PLA2 and acyltransferase were normal in the degenerating cerebellum of patients with spinocerebellar atrophy type 1, whereas the activity of glycerophosphocholine phosphodiesterase was reduced, suggesting that the alterations in AD brain were not nonspecific consequences of neurodegeneration. Our data suggest that compensatory phospholipid metabolic changes are present in AD brain that reduce the rate of phospholipid loss via both decreased catabolism (PLA2) and increased phospholipid resynthesis (acyltransferase and glycerophosphocholine phosphodiesterase).

PMID: 9453575

Panel 2.2 Summary: Ag1906 The expression of the CG56162-01 gene appears to be highest in a sample derived from normal kidney tissue (CT=28.1). In addition, there is substantial expression associated with kidney cancer tissue, liver cancer tissue, breast cancer tissue and colon cancer tissue. Thus, the expression of this gene could be used to distinguish this normal kidney tissue sample from other samples in the panel. Moreover, therapeutic modulation of thie gene, through the use of antibodies, small molecule drugs or protein therapeutics might be beneficial in the treatment of kidney cancer, liver cancer, breast cancer or colon cancer.

Panel 2D Summary: Ag1952 The expression of the CG56162-01 gene appears to be highest in a sample derived from kidney cancer tissue (CT=26.1). In addition, there is substantial expression associated with other samples of kidney cancer tissue, liver cancer tissue, breast cancer tissue, colon cancer tissue, normal prostate and normal lung. Thus, the expression of this gene could be used to distinguish this kidney cancer tissue sample from other samples in the panel. Moreover, therapeutic modulation of thie gene, through the use of

antibodies, small molecule drugs or protein therapeutics might be beneficial in the treatment of kidney cancer, liver cancer, breast cancer or colon cancer.

Panel 4D Summary: Ag1906/Ag1952/Ag2042 The expression of CG56162-01 gene was assessed in three independent runs using three different probe/primer pairs, with good concordance between the runs. This gene is expressed at moderate levels in a wide variety of cells including resting macrophages, TNF-alpha-activated dermal fibroblasts, LPS-stimulated dendritic cells, TNF-alpha+IL-1-beta-activated pulmonary artery endothelial cells, TNF-alpha+IL-1-beta-activated lung microvascular cells, and TNF-alpha+IFN-gamma-activated umbilical vein endothelial cells. Thus, sntibodies and small molecules that antagonize the function of the CG120803-01 geen product may be useful to reduce or eliminate the symptoms in patients with inflammatory and autoimmune diseases, such as lupus erythematosus, asthma, emphysema, Crohn's disease, ulcerative colitis, multiple sclerosis, rheumatoid arthritis, osteoarthritis, and psoriasis.

SEC10 (CG56164-01)

Expression of gene CG56164-01 was assessed using the primer-probe set Ag1677, described in Table 32A. Results of the RTQ-PCR runs are shown in Tables 32B, 32C, 23D, 32E and 32F.

Table 32A. Probe Name Ag1677

Primers	Sequences	Length	Start Position
Forward	5'-cccattcagcttcacagaga-3' (SEQ ID NO:189)	20	767
Probe	TET-5'-cagatcctggcattctctcagaagctg-3'-TAMRA (SEQ ID NO:190)	27	788
Reverse	5'-atgctcactgtctgttccttgt-3' (SEQ ID NO:191)	22	822

Table 32B. CNS_neurodegeneration_v1.0

Tissue Name	Rel. Exp.(%) Ag1677, Run 209733901	Tissue Name	Rel. Exp.(%) Ag1677, Run 209733901
AD 1 Hippo	9.9	Control (Path) 3 Temporal Ctx	8.2
AD 2 Hippo	16.7	Control (Path) 4 Temporal Ctx	75.8
AD 3 Hippo	5.3	AD 1 Occipital Ctx	14.4
AD 4 Hippo	26.2	AD 2 Occipital Ctx (Missing)	2.5
AD 5 Hippo	40.6	AD 3 Occipital Ctx	5.9
AD 6 Hippo	15.6	AD 4 Occipital Ctx	48.6
Control 2 Hippo	19.9	AD 5 Occipital Ctx	20.3
Control 4 Hippo	21.8	AD 6 Occipital Ctx	19.3
Control (Path) 3 Hippo	8.5	Control 1 Occipital Ctx	11.4
AD 1 Temporal Ctx	8.7	Control 2 Occipital Ctx	25.7
AD 2 Temporal Ctx	75.3	Control 3 Occipital Ctx	18.0

AD 3 Temporal Ctx	5.1	Control 4 Occipital Ctx	9.3
AD 4 Temporal Ctx	92.0	Control (Path) 1 Occipital Ctx	46.0
AD 5 Inf Temporal Ctx	**100.0**	Control (Path) 2 Occipital Ctx	48.0
AD 5 Sup Temporal Ctx	37.9	Control (Path) 3 Occipital Ctx	4.2
AD 6 Inf Temporal Ctx	19.5	Control (Path) 4 Occipital Ctx	7.8
AD 6 Sup Temporal Ctx	15.7	Control 1 Parietal Ctx	26.6
Control 1 Temporal Ctx	11.7	Control 2 Parietal Ctx	16.0
Control 2 Temporal Ctx	21.6	Control 3 Parietal Ctx	30.4
Control 3 Temporal Ctx	17.1	Control (Path) 1 Parietal Ctx	24.0
Control 3 Temporal Ctx	25.3	Control (Path) 2 Parietal Ctx	47.3
Control (Path) 1 Temporal Ctx	70.7	Control (Path) 3 Parietal Ctx	4.5
Control (Path) 2 Temporal Ctx	56.3	Control (Path) 4 Parietal Ctx	15.7

Table 32C. General_screening_panel_v1.4

Tissue Name	Rel. Exp.(%) Ag1677, Run 208021859	Tissue Name	Rel. Exp.(%) Ag1677, Run 208021859
Adipose	21.3	Renal ca. TK-10	0.6
Melanoma* Hs688(A).T	0.3	Bladder	6.3
Melanoma* Hs688(B).T	0.1	Gastric ca. (liver met.) NCI-N87	0.2
Melanoma* M14	0.1	Gastric ca. KATO III	0.1
Melanoma* LOXIMVI	0.2	Colon ca. SW-948	0.3
Melanoma* SK-MEL-5	0.7	Colon ca. SW480	0.2
Squamous cell carcinoma SCC-4	0.2	Colon ca.* (SW480 met) SW620	0.4
Testis Pool	4.7	Colon ca. HT29	1.3
Prostate ca.* (bone met) PC-3	0.3	Colon ca. HCT-116	0.4
Prostate Pool	3.0	Colon ca. CaCo-2	3.5
Placenta	6.3	Colon cancer tissue	1.9
Uterus Pool	2.0	Colon ca. SW1116	0.6
Ovarian ca. OVCAR-3	0.2	Colon ca. Colo-205	0.4
Ovarian ca. SK-OV-3	0.2	Colon ca. SW-48	0.4
Ovarian ca. OVCAR-4	0.3	Colon Pool	6.2
Ovarian ca. OVCAR-5	0.3	Small Intestine Pool	3.8
Ovarian ca. IGROV-1	0.6	Stomach Pool	3.8
Ovarian ca. OVCAR-8	0.8	Bone Marrow Pool	1.8
Ovary	1.8	Fetal Heart	19.1
Breast ca. MCF-7	24.0	Heart Pool	21.0
Breast ca. MDA-MB-231	0.4	Lymph Node Pool	4.7
Breast ca. BT 549	23.8	Fetal Skeletal Muscle	4.4
Breast ca. T47D	0.7	Skeletal Muscle Pool	14.0

Breast ca. MDA-N	0.7	Spleen Pool	2.5
Breast Pool	3.6	Thymus Pool	3.3
Trachea	2.7	CNS cancer (glio/astro) U87-MG	0.1
Lung	2.9	CNS cancer (glio/astro) U-118-MG	1.0
Fetal Lung	82.4	CNS cancer (neuro;met) SK-N-AS	0.1
Lung ca. NCI-N417	0.1	CNS cancer (astro) SF-539	0.2
Lung ca. LX-1	0.2	CNS cancer (astro) SNB-75	0.7
Lung ca. NCI-H146	0.2	CNS cancer (glio) SNB-19	1.3
Lung ca. SHP-77	31.6	CNS cancer (glio) SF-295	0.2
Lung ca. A549	0.9	Brain (Amygdala) Pool	12.1
Lung ca. NCI-H526	0.5	Brain (cerebellum)	**100.0**
Lung ca. NCI-H23	0.3	Brain (fetal)	3.0
Lung ca. NCI-H460	0.2	Brain (Hippocampus) Pool	8.4
Lung ca. HOP-62	0.2	Cerebral Cortex Pool	21.2
Lung ca. NCI-H522	0.0	Brain (Substantia nigra) Pool	22.7
Liver	0.4	Brain (Thalamus) Pool	14.5
Fetal Liver	0.7	Brain (whole)	22.1
Liver ca. HepG2	0.3	Spinal Cord Pool	5.3
Kidney Pool	14.8	Adrenal Gland	2.9
Fetal Kidney	19.6	Pituitary gland Pool	10.4
Renal ca. 786-0	0.4	Salivary Gland	5.1
Renal ca. A498	0.5	Thyroid (female)	39.5
Renal ca. ACHN	0.2	Pancreatic ca. CAPAN2	0.4
Renal ca. UO-31	0.2	Pancreas Pool	6.1

Table 32D. Panel 1.3D

Tissue Name	Rel. Exp.(%) Ag1677, Run 152171910	Rel. Exp.(%) Ag1677, Run 165532764	Tissue Name	Rel. Exp.(%) Ag1677, Run 152171910	Rel. Exp.(%) Ag1677, Run 165532764
Liver adenocarcinoma	0.0	0.1	Kidney (fetal)	1.9	7.0
Pancreas	14.8	23.5	Renal ca. 786-0	0.0	0.0
Pancreatic ca. CAPAN 2	0.0	0.1	Renal ca. A498	0.0	0.2
Adrenal gland	2.1	3.3	Renal ca. RXF 393	0.0	1.0
Thyroid	36.1	37.1	Renal ca. ACHN	0.0	0.5
Salivary gland	3.2	10.0	Renal ca. UO-31	0.0	0.0
Pituitary gland	6.4	10.4	Renal ca. TK-10	0.0	0.3
Brain (fetal)	0.8	1.6	Liver	0.0	0.9
Brain (whole)	13.2	36.6	Liver (fetal)	0.0	1.9
Brain (amygdala)	4.6	17.9	Liver ca. (hepatoblast) HepG2	0.0	0.0
Brain (cerebellum)	11.1	74.2	Lung	**100.0**	**100.0**
Brain (hippocampus)	23.8	30.6	Lung (fetal)	59.5	72.2
Brain (substantia nigra)	4.6	18.7	Lung ca. (small cell) LX-1	0.0	1.1

Brain (thalamus)	3.5	20.2	Lung ca. (small cell) NCI-H69	0.0	0.2
Cerebral Cortex	22.4	28.3	Lung ca. (s.cell var.) SHP-77	0.0	0.7
Spinal cord	0.9	6.9	Lung ca. (large cell)NCI-H460	0.0	0.7
glio/astro U87-MG	0.0	0.1	Lung ca. (non-sm. cell) A549	0.0	0.9
glio/astro U-118-MG	0.0	2.2	Lung ca. (non-s.cell) NCI-H23	0.2	1.0
astrocytoma SW1783	0.0	0.7	Lung ca. (non-s.cell) HOP-62	0.0	0.3
neuro*; met SK-N-AS	0.0	0.5	Lung ca. (non-s.cl) NCI-H522	0.0	0.8
astrocytoma SF-539	0.1	0.7	Lung ca. (squam.) SW 900	0.0	0.7
astrocytoma SNB-75	0.0	1.0	Lung ca. (squam.) NCI-H596	0.0	0.6
glioma SNB-19	0.0	1.0	Mammary gland	16.7	19.5
glioma U251	0.0	1.1	Breast ca.* (pl.ef) MCF-7	3.8	5.3
glioma SF-295	0.0	1.2	Breast ca.* (pl.ef) MDA-MB-231	0.0	0.2
Heart (fetal)	55.5	40.6	Breast ca.* (pl.ef) T47D	0.0	0.7
Heart	11.9	47.0	Breast ca. BT-549	0.4	2.2
Skeletal muscle (fetal)	41.2	22.7	Breast ca. MDA-N	0.0	0.9
Skeletal muscle	2.0	22.4	Ovary	3.5	3.1
Bone marrow	4.8	12.1	Ovarian ca. OVCAR-3	0.0	1.3
Thymus	0.4	1.9	Ovarian ca. OVCAR-4	0.0	0.0
Spleen	2.3	3.2	Ovarian ca. OVCAR-5	0.0	0.6
Lymph node	4.2	15.7	Ovarian ca. OVCAR-8	0.0	1.4
Colorectal	57.4	64.2	Ovarian ca. IGROV-1	0.0	0.3
Stomach	11.6	20.9	Ovarian ca.* (ascites) SK-OV-3	0.0	0.7
Small intestine	4.0	7.5	Uterus	2.5	19.6
Colon ca. SW480	0.0	0.1	Placenta	3.7	4.3
Colon ca.* SW620(SW480 met)	0.0	0.2	Prostate	4.5	8.4
Colon ca. HT29	0.0	0.2	Prostate ca.* (bone met)PC-3	0.0	0.2
Colon ca. HCT-116	0.0	0.9	Testis	0.5	3.2
Colon ca. CaCo-2	0.4	1.5	Melanoma Hs688(A).T	0.0	0.7

Colon ca. tissue(ODO3866)	0.9	3.0	Melanoma* (met) Hs688(B).T	0.0	0.0
Colon ca. HCC-2998	0.5	2.4	Melanoma UACC-62	0.0	0.4
Gastric ca.* (liver met) NCI-N87	0.0	0.6	Melanoma M14	0.0	0.2
Bladder	0.0	2.7	Melanoma LOX IMVI	0.0	0.6
Trachea	2.4	6.6	Melanoma* (met) SK-MEL-5	0.0	0.6
Kidney	9.6	37.9	Adipose	7.2	15.3

Table 32E. Panel 2D

Tissue Name	Rel. Exp.(%) Ag1677, Run 152570595	Tissue Name	Rel. Exp.(%) Ag1677, Run 152570595
Normal Colon	63.3	Kidney Margin 8120608	26.6
CC Well to Mod Diff (ODO3866)	0.5	Kidney Cancer 8120613	2.3
CC Margin (ODO3866)	77.9	Kidney Margin 8120614	50.0
CC Gr.2 rectosigmoid (ODO3868)	5.3	Kidney Cancer 9010320	0.7
CC Margin (ODO3868)	2.2	Kidney Margin 9010321	26.2
CC Mod Diff (ODO3920)	29.5	Normal Uterus	0.6
CC Margin (ODO3920)	**100.0**	Uterus Cancer 064011	1.9
CC Gr.2 ascend colon (ODO3921)	30.6	Normal Thyroid	21.9
CC Margin (ODO3921)	42.9	Thyroid Cancer 064010	0.6
CC from Partial Hepatectomy (ODO4309) Mets	1.0	Thyroid Cancer A302152	1.1
Liver Margin (ODO4309)	0.0	Thyroid Margin A302153	14.5
Colon mets to lung (OD04451-01)	1.5	Normal Breast	6.6
Lung Margin (OD04451-02)	15.5	Breast Cancer (OD04566)	0.1
Normal Prostate 6546-1	3.3	Breast Cancer (OD04590-01)	0.6
Prostate Cancer (OD04410)	1.8	Breast Cancer Mets (OD04590-03)	5.1
Prostate Margin (OD04410)	1.4	Breast Cancer Metastasis (OD04655-05)	0.7
Prostate Cancer (OD04720-01)	0.5	Breast Cancer 064006	0.1
Prostate Margin (OD04720-02)	1.3	Breast Cancer 1024	5.6
Normal Lung 061010	36.3	Breast Cancer 9100266	0.3
Lung Met to Muscle (ODO4286)	0.1	Breast Margin 9100265	0.7
Muscle Margin (ODO4286)	2.7	Breast Cancer A209073	0.3
Lung Malignant Cancer (OD03126)	6.1	Breast Margin A2090734	1.3
Lung Margin (OD03126)	63.7	Normal Liver	0.0
Lung Cancer (OD04404)	3.5	Liver Cancer 064003	0.1
Lung Margin (OD04404)	17.3	Liver Cancer 1025	0.0
Lung Cancer (OD04565)	0.0	Liver Cancer 1026	0.0
Lung Margin (OD04565)	21.3	Liver Cancer 6004-T	0.2

Lung Cancer (OD04237-01)	0.2	Liver Tissue 6004-N	0.0
Lung Margin (OD04237-02)	17.9	Liver Cancer 6005-T	0.2
Ocular Mel Met to Liver (ODO4310)	0.1	Liver Tissue 6005-N	0.0
Liver Margin (ODO4310)	0.0	Normal Bladder	1.3
Melanoma Mets to Lung (OD04321)	0.1	Bladder Cancer 1023	0.0
Lung Margin (OD04321)	33.9	Bladder Cancer A302173	0.0
Normal Kidney	18.4	Bladder Cancer (OD04718-01)	0.2
Kidney Ca, Nuclear grade 2 (OD04338)	0.8	Bladder Normal Adjacent (OD04718-03)	0.6
Kidney Margin (OD04338)	19.3	Normal Ovary	2.3
Kidney Ca Nuclear grade 1/2 (OD04339)	0.1	Ovarian Cancer 064008	0.0
Kidney Margin (OD04339)	50.7	Ovarian Cancer (OD04768-07)	0.6
Kidney Ca, Clear cell type (OD04340)	14.9	Ovary Margin (OD04768-08)	0.1
Kidney Margin (OD04340)	34.4	Normal Stomach	1.9
Kidney Ca, Nuclear grade 3 (OD04348)	0.3	Gastric Cancer 9060358	0.2
Kidney Margin (OD04348)	13.9	Stomach Margin 9060359	0.8
Kidney Cancer (OD04622-01)	0.2	Gastric Cancer 9060395	0.1
Kidney Margin (OD04622-03)	4.8	Stomach Margin 9060394	1.2
Kidney Cancer (OD04450-01)	0.0	Gastric Cancer 9060397	3.9
Kidney Margin (OD04450-03)	15.0	Stomach Margin 9060396	0.5
Kidney Cancer 8120607	0.1	Gastric Cancer 064005	0.1

Table 32F. Panel 4D

Tissue Name	Rel. Exp.(%) Ag1677, Run 152571252	Tissue Name	Rel. Exp.(%) Ag1677, Run 152571252
Secondary Th1 act	0.0	HUVEC IL-1beta	0.0
Secondary Th2 act	0.0	HUVEC IFN gamma	0.0
Secondary Tr1 act	0.0	HUVEC TNF alpha + IFN gamma	0.0
Secondary Th1 rest	0.0	HUVEC TNF alpha + IL4	0.0
Secondary Th2 rest	0.0	HUVEC IL-11	1.0
Secondary Tr1 rest	0.0	Lung Microvascular EC none	0.0
Primary Th1 act	0.0	Lung Microvascular EC TNFalpha + IL-1beta	0.0
Primary Th2 act	0.0	Microvascular Dermal EC none	0.5
Primary Tr1 act	0.0	Microsvasular Dermal EC TNFalpha + IL-1beta	0.0
Primary Th1 rest	0.0	Bronchial epithelium TNFalpha + IL1beta	0.0
Primary Th2 rest	0.0	Small airway epithelium none	0.0
Primary Tr1 rest	0.0	Small airway epithelium TNFalpha + IL-1beta	0.0
CD45RA CD4 lymphocyte act	0.0	Coronery artery SMC rest	0.0
CD45RO CD4 lymphocyte	0.0	Coronery artery SMC TNFalpha	0.0

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act		+ IL-1beta	
CD8 lymphocyte act	0.0	Astrocytes rest	0.0
Secondary CD8 lymphocyte rest	0.0	Astrocytes TNFalpha + IL-1beta	0.8
Secondary CD8 lymphocyte act	0.0	KU-812 (Basophil) rest	1.4
CD4 lymphocyte none	0.0	KU-812 (Basophil) PMA/ionomycin	0.0
2ry Th1/Th2/Tr1_anti-CD95 CH11	0.0	CCD1106 (Keratinocytes) none	0.0
LAK cells rest	0.0	CCD1106 (Keratinocytes) TNFalpha + IL-1beta	0.0
LAK cells IL-2	0.0	Liver cirrhosis	0.0
LAK cells IL-2+IL-12	0.0	Lupus kidney	10.5
LAK cells IL-2+IFN gamma	0.4	NCI-H292 none	0.0
LAK cells IL-2+ IL-18	0.0	NCI-H292 IL-4	0.0
LAK cells PMA/ionomycin	0.0	NCI-H292 IL-9	0.0
NK Cells IL-2 rest	0.0	NCI-H292 IL-13	0.0
Two Way MLR 3 day	0.0	NCI-H292 IFN gamma	0.0
Two Way MLR 5 day	0.0	HPAEC none	0.0
Two Way MLR 7 day	0.0	HPAEC TNF alpha + IL-1 beta	0.0
PBMC rest	0.0	Lung fibroblast none	0.0
PBMC PWM	0.0	Lung fibroblast TNF alpha + IL-1 beta	0.0
PBMC PHA-L	0.0	Lung fibroblast IL-4	0.0
Ramos (B cell) none	0.8	Lung fibroblast IL-9	0.0
Ramos (B cell) ionomycin	0.4	Lung fibroblast IL-13	0.0
B lymphocytes PWM	0.0	Lung fibroblast IFN gamma	0.0
B lymphocytes CD40L and IL-4	0.0	Dermal fibroblast CCD1070 rest	0.0
EOL-1 dbcAMP	0.0	Dermal fibroblast CCD1070 TNF alpha	0.0
EOL-1 dbcAMP PMA/ionomycin	0.0	Dermal fibroblast CCD1070 IL-1 beta	0.0
Dendritic cells none	0.0	Dermal fibroblast IFN gamma	0.0
Dendritic cells LPS	0.0	Dermal fibroblast IL-4	5.8
Dendritic cells anti-CD40	0.0	IBD Colitis 2	0.5
Monocytes rest	0.0	IBD Crohn's	0.0
Monocytes LPS	0.0	Colon	9.7
Macrophages rest	0.0	Lung	14.1
Macrophages LPS	0.0	Thymus	**100.0**
HUVEC none	0.0	Kidney	3.9
HUVEC starved	0.0		

CNS_neurodegeneration_v1.0 Summary: Ag1677 No change of expression of the CG56164-01 gene is noted in Alzheimer's disease, consistent with the scientific literature.

However, this panel does confirm expression of this gene in the brain. Please see Panel 1.3D for discussion of utility of this gene in the central nervous system.

References:

Vlkolinsky R, Cairns N, Fountoulakis M, Lubec G. Decreased brain levels of 2',3'-cyclic nucleotide-3'-phosphodiesterase in Down syndrome and Alzheimer's disease. Neurobiol Aging 2001 Jul-Aug;22(4):547-53

In Down syndrome (DS) as well as in Alzheimer's disease (AD) oligodendroglial and myelin alterations have been reported. 2',3'-cyclic nucleotide-3'-phosphodiesterase (CNPase) and carbonic anhydrase II (CA II) are widely accepted as markers for oligodendroglia and myelin. However, only data on CNPase activity have been available in AD and DS brains so far. In our study we determined the protein levels of CNPase and CA II in DS, AD and in control post mortem brain samples in order to assess oligodendroglia and myelin alterations in both diseases. We used two dimensional electrophoresis to separate brain proteins that were subsequently identified by matrix assisted laser desorption and ionization mass-spectroscopy (MALDI-MS). Seven brain areas were investigated (frontal, temporal, occipital and parietal cortex, cerebellum, thalamus and caudate nucleus). In comparison to control brains we detected significantly decreased CNPase protein levels in frontal and temporal cortex of DS patients. The level of CA II protein in DS was unchanged in comparison to controls. In AD brains levels of CNPase were decreased in frontal cortex only. The level of CA II in all brain areas in AD group was comparable to controls. Changes of CNPase protein levels in DS and AD are in agreement with the previous finding of decreased CNPase activity in DS and AD brain. They probably reflect decreased oligodendroglial density and/or reduced myelination. These can be secondary to disturbances in axon/oligodendroglial communication due to neuronal loss present in both diseases. Alternatively, reduced CNPase levels in DS brains may be caused by impairment of glucose metabolism and/or alterations of thyroid functions.

General_screening_panel_v1.4 Summary: Ag1677 Highest expression of the CG56164-01 gene in this panel is seen in the cerebellum (CT=26.2), with expression also seen across all brain areas represented in this panel. This expression profile is consistent with the brain expression seen in the CNS_neurodegeneration_v1.0 panel. Please see Panel 1.3D for discussion of utility of this gene in the central nervous system.

Overall, this gene is expressed mostly in normal tissues, with much lower expression in most cancer cell lines. This suggests that loss of expression of this gene might be required for the proliferation of these cancer cell lines. A moderate level of expression is seen in a lung

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cancer and two breast cancer cell lines. Thus, the loss of expression might be used as a diagnostic marker for most cancers, except the cancer tissues from which the lung and breast cancer cell lines were derived. In addition, the protein product of this gene might be of use in the treatment of these cancers.

5   This gene is also moderately expressed in a wide variety of metabolic tissues including pancreas, adrenal, thyroid, pituitary, adult and fetal heart, adult and fetal skeletal muscle, adult and fetal liver, and adipose. Carbonic anhydrase III is reduced in adipose tissue in several animal models of genetic obesity. Thus, an activator of this gene product could potentially be a drug treatment for the prevention and/or treatment of obesity in humans.

10   In addition, this gene is expressed at higher levels in fetal lung (CT=26.5) than in adult lung (CT=31.3). Thus, expression of this gene could be used to differentiate between fetal and adult lung tissue. The expression of this gene at significant levels in the lung is consistent with published reports (see references below.) This suggests that the gene product is involved in the homeostasis of the lung. Therefore, therapeutic modulation of the expression or function of the

15   protein encoded by this gene could be effective in treating disease that affect the lung or its function.

Reference:

Takahashi H, et al. Detection and identification of subcutaneous adipose tissue proteins related to obesity in New Zealand obese mouse. Endocr J 48:205-11, 2001.

20   New Zealand obese (NZO) mouse, a genetic model of obesity, shows hyperphagia, hyperinsulinemia and leptin resistance. We analyzed subcutaneous adipose tissue proteins in NZO mice with a two-dimensional gel electrophoresis technique followed by protein sequence analysis. NZO mice showed hyperinsulinemia and hyperleptinemia. Abdominal subcutaneous adipose tissue was inspected in NZO and C57BL/6J lean mice. Two-dimensional gel

25   electrophoresis detected 4 spots which were obviously reduced in NZO mice. Those spots were p26, p19, p18 and p15. Internal sequences of the p26 and p15 protein were homologous with those of carbonic anhydrase III, p19 was cytochrome b5, p18 was superoxide dismutase. Serum arachidonic acid level in NZO mice was lower by 80% of C57BL/6J mice. The present study demonstrated the reduction of several enzymes related to lipid metabolism in NZO mice.

30   These data raises the hypothesis that the supposed changes of membrane fluidity caused by altered membrane lipid content may involve central leptin resistance of this model of obesity.

PMID: 11456269

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Stanton LW, et al. Expression of CAIII in rodent models of obesity. Mol Endocrinol 5:860-6, 1991.

To achieve a better understanding of the biochemical basis of obesity, we have undertaken comparative analyses of adipose tissue of lean and obese mice. By two-dimensional gel analysis, carbonic anhydrase-III (CA III) has been identified as a major constituent of murine adipose tissue. Quantitative comparisons of CA III protein and mRNA levels indicate that this enzyme is expressed at lower levels in adipose tissue from animals that were either genetically obese or had experimentally induced obesity compared to levels in the corresponding lean controls. This decrease in CA III expression was unique to adipose tissue, since other CA III-containing organs and tissues did not show a change when lean and obese animals were compared. Additionally, levels of CA III in adipose tissue from obese animals responded to acute changes in energy balance of the animal. These results are discussed in light of possible metabolic roles for CA III.

PMID: 1922100

Mahieu I, Benjamin A, Stephens R, Walters D, Carter N.Characterization of membrane bound carbonic anhydrase IV (CA IV) located on the external surface of lung pulmonary endothelial cells. Biochem Soc Trans 1995 May;23(2):320S

PMID: 7672351

Panel 1.3D Summary: Ag1677 The expression of the CG56164-01 gene was assessed in two independent runs on this panel and there appears to be good concordance between runs. This gene is expressed mostly innormal tissues, with much lower expression in most cancer cell lines. Highest expression of the gene in this panel is seen in the lung (CTs=28). This significant expression in the lung is consistent with the results in General_screening_panel_v1.4 and suggests that this gene product is involved in the homeostasis of this organ. The higher association of this gene with normal tissues suggests that loss of expression of this gene might be required for the proliferation of the cancer cell lines in this panel. Thus, this loss of expression might be used as a diagnostic marker for cancer.

As in the previous panel, this gene is widely expressed in a variety of metabolic tissues including pancreas, adrenal, thyroid, pituitary, adult and fetal heart, adult and fetal skeletal muscle, and adipose. Thus, this gene product may be a small molecule target for the treatment of metabolic disease, including Types 1 and 2 diabetes.

This gene encodes a homolog of carbonic anhydrase which is a known marker for oligodendroglia. Carbonic anhydrase expression in the brain is useful for distinguishing between neurons and oligodendroglia. Thus, this gene product may utility in monitoring the progression of diseases that involve the myelinating function of oligodendroglia, such as Multiple Sclerosis and Alzheimer's disease.

Panel 2D Summary: Ag1677 As in the previous panels, expression of the CG56164-01 gene is more highly associated with normal tissues. Highest expression of the gene in this panel is seen in a normal colon sample (CT=27.8). Furthermore, expression of this gene is higher in normal colon, stomach, ovary, thyroid, kidney and lung than in the corresponding adjacent tumor tissues. Thus, the loss of expression of this gene could be used to distinguish malignant colon, lung, stomach, ovary, thyroid and kidney tissue from normal tissue from these organs. In addition, the protein product of this gene might be of use in the treatment of these cancers.

Panel 4D Summary: Ag1677 The CG55936-01 transcript is expressed at low but significant levels in the thymus, lung and kidney (CTs=30-35), again showing preferential expression in normal tissues. Thus, this gene or the protein it encodes could be used to detect these tissues. Therapeutically, the protein encoded for by this transcript could be used for immune modulation by regulating T cell development in the thymus.

NOV7 (CG55117-01)

Expression of gene CG55117-01 was assessed using the primer-probe set Ag819, described in Table 33A. Results of the RTQ-PCR runs are shown in Tables 33B, 33C, 33D, 33E and 33F.

Table 33A. Probe Name Ag819

Primers	Sequences	Length	Start Position
Forward	5'-ggtccaacagggctatcaat-3' (SEQ ID NO:192)	20	1136
Probe	TET-5'-ccaaaccacgactgtcgtagcaggta-3'-TAMRA (SEQ ID NO:193)	26	1187
Reverse	5'-tggaattcaagaccctttga-3' (SEQ ID NO:194)	21	1213

Table 33B. Panel 1.2

Tissue Name	Rel. Exp.(%) Ag819, Run 118349021	Rel. Exp.(%) Ag819, Run 120989791	Tissue Name	Rel. Exp.(%) Ag819, Run 118349021	Rel. Exp.(%) Ag819, Run 120989791
Endothelial cells	0.0	0.0	Renal ca. 786-0	0.0	0.0
Heart (Fetal)	0.4	0.8	Renal ca. A498	0.0	0.1
Pancreas	43.8	48.3	Renal ca. RXF 393	0.0	0.0
Pancreatic ca.	8.1	17.9	Renal ca.	0.0	0.0

CAPAN 2			ACHN		
Adrenal Gland	0.2	0.2	Renal ca. UO-31	1.0	1.6
Thyroid	11.8	12.9	Renal ca. TK-10	0.0	0.0
Salivary gland	63.3	63.7	Liver	8.0	3.3
Pituitary gland	0.9	0.5	Liver (fetal)	2.8	2.7
Brain (fetal)	37.1	41.5	Liver ca. (hepatoblast) HepG2	12.8	20.2
Brain (whole)	4.6	6.3	Lung	5.7	4.2
Brain (amygdala)	1.5	2.3	Lung (fetal)	9.5	7.4
Brain (cerebellum)	0.9	1.5	Lung ca. (small cell) LX-1	39.0	33.4
Brain (hippocampus)	3.4	4.0	Lung ca. (small cell) NCI-H69	7.4	10.5
Brain (thalamus)	1.9	2.6	Lung ca. (s.cell var.) SHP-77	0.5	0.6
Cerebral Cortex	1.2	2.7	Lung ca. (large cell)NCI-H460	0.0	0.0
Spinal cord	1.2	1.9	Lung ca. (non-sm. cell) A549	0.0	0.1
glio/astro U87-MG	0.0	0.0	Lung ca. (non-s.cell) NCI-H23	0.0	0.0
glio/astro U-118-MG	0.0	0.0	Lung ca. (non-s.cell) HOP-62	0.1	0.2
astrocytoma SW1783	0.0	0.0	Lung ca. (non-s.cl) NCI-H522	0.0	0.1
neuro*; met SK-N-AS	0.3	0.1	Lung ca. (squam.) SW 900	0.6	0.8
astrocytoma SF-539	0.0	0.0	Lung ca. (squam.) NCI-H596	14.6	22.1
astrocytoma SNB-75	0.0	0.0	Mammary gland	33.0	46.3
glioma SNB-19	0.0	0.1	Breast ca.* (pl.ef) MCF-7	0.0	0.0
glioma U251	0.0	0.1	Breast ca.* (pl.ef) MDA-MB-231	0.0	0.0
glioma SF-295	0.0	0.0	Breast ca.* (pl. ef) T47D	0.8	1.3
Heart	8.1	9.5	Breast ca. BT-	0.0	0.0

			549		
Skeletal Muscle	2.6	3.7	Breast ca. MDA-N	0.4	0.6
Bone marrow	0.6	1.2	Ovary	4.6	0.2
Thymus	0.0	0.1	Ovarian ca. OVCAR-3	3.3	4.0
Spleen	0.5	0.0	Ovarian ca. OVCAR-4	27.9	54.0
Lymph node	1.4	0.2	Ovarian ca. OVCAR-5	37.4	51.1
Colorectal Tissue	0.3	1.8	Ovarian ca. OVCAR-8	0.0	0.1
Stomach	10.7	23.3	Ovarian ca. IGROV-1	3.2	5.5
Small intestine	10.4	18.9	Ovarian ca. (ascites) SK-OV-3	0.0	0.0
Colon ca. SW480	0.0	0.0	Uterus	1.4	1.2
Colon ca.* SW620 (SW480 met)	9.0	11.7	Placenta	23.2	22.5
Colon ca. HT29	32.5	40.9	Prostate	2.6	2.7
Colon ca. HCT-116	5.9	7.9	Prostate ca.* (bone met) PC-3	0.0	0.0
Colon ca. CaCo-2	**100.0**	**100.0**	Testis	19.8	21.9
Colon ca. Tissue (ODO3866)	4.7	5.4	Melanoma Hs688(A).T	1.7	0.0
Colon ca. HCC-2998	2.5	3.0	Melanoma* (met) Hs688(B).T	0.7	0.0
Gastric ca.* (liver met) NCI-N87	0.0	0.2	Melanoma UACC-62	1.8	1.7
Bladder	39.2	49.7	Melanoma M14	0.1	0.2
Trachea	29.7	34.4	Melanoma LOX IMVI	0.0	0.0
Kidney	27.4	25.7	Melanoma* (met) SK-MEL-5	0.5	1.0
Kidney (fetal)	17.7	19.1			

Table 33C. Panel 2D

Tissue Name	Rel. Exp.(%)	Rel. Exp.(%)	Tissue Name	Rel. Exp.(%)	Rel. Exp.(%)

	Ag819, Run 144794769	Ag819, Run 146791894		Ag819, Run 144794769	Ag819, Run 146791894
Normal Colon	17.0	20.7	Kidney Margin 8120608	3.9	2.0
CC Well to Mod Diff (ODO3866)	0.9	5.3	Kidney Cancer 8120613	0.1	0.0
CC Margin (ODO3866)	9.5	6.0	Kidney Margin 8120614	1.2	0.8
CC Gr.2 rectosigmoid (ODO3868)	5.8	3.9	Kidney Cancer 9010320	7.7	8.0
CC Margin (ODO3868)	0.0	0.2	Kidney Margin 9010321	7.8	6.0
CC Mod Diff (ODO3920)	0.7	0.9	Normal Uterus	0.0	0.0
CC Margin (ODO3920)	2.8	2.1	Uterus Cancer 064011	24.1	18.8
CC Gr.2 ascend colon (ODO3921)	26.2	37.4	Normal Thyroid	4.7	2.4
CC Margin (ODO3921)	4.4	7.0	Thyroid Cancer 064010	4.0	2.2
CC from Partial Hepatectomy (ODO4309) Mets	13.1	20.4	Thyroid Cancer A302152	0.1	0.0
Liver Margin (ODO4309)	0.1	0.2	Thyroid Margin A302153	2.9	2.7
Colon mets to lung (OD04451-01)	8.5	6.2	Normal Breast	16.6	7.4
Lung Margin (OD04451-02)	2.1	1.7	Breast Cancer (OD04566)	0.6	0.4
Normal Prostate 6546-1	1.2	0.3	Breast Cancer (OD04590-01)	0.8	0.5
Prostate Cancer (OD04410)	0.5	0.7	Breast Cancer Mets (OD04590-03)	0.0	0.0
Prostate Margin (OD04410)	0.8	0.6	Breast Cancer Metastasis (OD04655-05)	0.1	0.0
Prostate Cancer (OD04720-01)	0.4	0.4	Breast Cancer 064006	15.7	11.7
Prostate Margin	2.0	2.0	Breast Cancer	12.1	11.6

(OD04720-02)			1024		
Normal Lung 061010	4.6	4.7	Breast Cancer 9100266	1.2	0.6
Lung Met to Muscle (ODO4286)	0.0	0.0	Breast Margin 9100265	3.0	2.0
Muscle Margin (ODO4286)	0.2	0.3	Breast Cancer A209073	6.5	4.6
Lung Malignant Cancer (OD03126)	8.7	6.5	Breast Margin A2090734	25.0	9.0
Lung Margin (OD03126)	1.4	1.6	Normal Liver	0.6	0.5
Lung Cancer (OD04404)	0.1	0.2	Liver Cancer 064003	0.0	0.0
Lung Margin (OD04404)	3.1	1.2	Liver Cancer 1025	0.2	0.3
Lung Cancer (OD04565)	0.2	0.1	Liver Cancer 1026	0.2	0.1
Lung Margin (OD04565)	1.0	0.9	Liver Cancer 6004-T	0.2	0.1
Lung Cancer (OD04237-01)	**100.0**	74.7	Liver Tissue 6004-N	1.6	1.7
Lung Margin (OD04237-02)	1.7	1.5	Liver Cancer 6005-T	0.1	0.2
Ocular Mel Met to Liver (ODO4310)	0.1	0.3	Liver Tissue 6005-N	0.0	0.2
Liver Margin (ODO4310)	0.6	0.2	Normal Bladder	14.8	18.7
Melanoma Mets to Lung (OD04321)	14.8	12.2	Bladder Cancer 1023	6.9	6.4
Lung Margin (OD04321)	1.7	1.5	Bladder Cancer A302173	0.2	0.1
Normal Kidney	23.2	17.4	Bladder Cancer (OD04718-01)	0.1	0.1
Kidney Ca, Nuclear grade 2 (OD04338)	4.2	5.1	Bladder Normal Adjacent (OD04718-03)	0.2	0.4
Kidney Margin (OD04338)	8.0	11.3	Normal Ovary	0.1	0.0
Kidney Ca Nuclear grade	65.5	69.3	Ovarian Cancer 064008	68.8	**100.0**

1/2 (OD04339)					
Kidney Margin (OD04339)	6.7	6.0	Ovarian Cancer (OD04768-07)	0.5	1.0
Kidney Ca, Clear cell type (OD04340)	0.1	0.1	Ovary Margin (OD04768-08)	0.0	0.1
Kidney Margin (OD04340)	13.8	12.0	Normal Stomach	5.0	4.5
Kidney Ca, Nuclear grade 3 (OD04348)	0.0	0.0	Gastric Cancer 9060358	2.5	2.6
Kidney Margin (OD04348)	9.2	6.3	Stomach Margin 9060359	5.6	7.0
Kidney Cancer (OD04622-01)	0.7	0.4	Gastric Cancer 9060395	1.7	1.3
Kidney Margin (OD04622-03)	1.1	1.2	Stomach Margin 9060394	3.4	6.4
Kidney Cancer (OD04450-01)	32.5	24.5	Gastric Cancer 9060397	26.1	39.8
Kidney Margin (OD04450-03)	22.1	16.0	Stomach Margin 9060396	2.7	2.7
Kidney Cancer 8120607	4.4	4.2	Gastric Cancer 064005	15.5	22.1

Table 33D. Panel 3D

Tissue Name	Rel. Exp.(%) Ag819, Run 172133330	Tissue Name	Rel. Exp.(%) Ag819, Run 172133330
Daoy- Medulloblastoma	0.9	Ca Ski- Cervical epidermoid carcinoma (metastasis)	0.0
TE671- Medulloblastoma	1.1	ES-2- Ovarian clear cell carcinoma	0.0
D283 Med- Medulloblastoma	**100.0**	Ramos- Stimulated with PMA/ionomycin 6h	0.0
PFSK-1- Primitive Neuroectodermal	0.0	Ramos- Stimulated with PMA/ionomycin 14h	0.0
XF-498- CNS	0.1	MEG-01- Chronic myelogenous leukemia (megokaryoblast)	0.0
SNB-78- Glioma	0.0	Raji- Burkitt's lymphoma	0.0
SF-268- Glioblastoma	0.0	Daudi- Burkitt's lymphoma	0.0
T98G- Glioblastoma	0.0	U266- B-cell plasmacytoma	0.0
SK-N-SH-	0.0	CA46- Burkitt's lymphoma	0.0

Neuroblastoma (metastasis)			
SF-295- Glioblastoma	0.0	RL- non-Hodgkin's B-cell lymphoma	0.0
Cerebellum	0.8	JM1- pre-B-cell lymphoma	0.0
Cerebellum	0.2	Jurkat- T cell leukemia	0.0
NCI-H292- Mucoepidermoid lung carcinoma	0.0	TF-1- Erythroleukemia	0.0
DMS-114- Small cell lung cancer	0.1	HUT 78- T-cell lymphoma	0.0
DMS-79- Small cell lung cancer	9.9	U937- Histiocytic lymphoma	0.0
NCI-H146- Small cell lung cancer	1.2	KU-812- Myelogenous leukemia	0.0
NCI-H526- Small cell lung cancer	0.0	769-P- Clear cell renal carcinoma	0.0
NCI-N417- Small cell lung cancer	0.0	Caki-2- Clear cell renal carcinoma	0.0
NCI-H82- Small cell lung cancer	28.5	SW 839- Clear cell renal carcinoma	0.0
NCI-H157- Squamous cell lung cancer (metastasis)	0.0	G401- Wilms' tumor	0.0
NCI-H1155- Large cell lung cancer	3.4	Hs766T- Pancreatic carcinoma (LN metastasis)	0.0
NCI-H1299- Large cell lung cancer	0.0	CAPAN-1- Pancreatic adenocarcinoma (liver metastasis)	9.0
NCI-H727- Lung carcinoid	0.4	SU86.86- Pancreatic carcinoma (liver metastasis)	17.6
NCI-UMC-11- Lung carcinoid	10.4	BxPC-3- Pancreatic adenocarcinoma	0.0
LX-1- Small cell lung cancer	27.9	HPAC- Pancreatic adenocarcinoma	1.7
Colo-205- Colon cancer	3.0	MIA PaCa-2- Pancreatic carcinoma	0.0
KM12- Colon cancer	3.3	CFPAC-1- Pancreatic ductal adenocarcinoma	58.2
KM20L2- Colon cancer	1.2	PANC-1- Pancreatic epithelioid ductal carcinoma	0.0
NCI-H716- Colon cancer	0.0	T24- Bladder carcinma (transitional cell)	0.0
SW-48- Colon adenocarcinoma	7.3	5637- Bladder carcinoma	0.0
SW1116- Colon	3.7	HT-1197- Bladder carcinoma	0.0

adenocarcinoma			
LS 174T- Colon adenocarcinoma	0.2	UM-UC-3- Bladder carcinma (transitional cell)	0.0
SW-948- Colon adenocarcinoma	0.0	A204- Rhabdomyosarcoma	0.0
SW-480- Colon adenocarcinoma	3.1	HT-1080- Fibrosarcoma	0.0
NCI-SNU-5- Gastric carcinoma	0.0	MG-63- Osteosarcoma	0.0
KATO III- Gastric carcinoma	0.0	SK-LMS-1- Leiomyosarcoma (vulva)	0.0
NCI-SNU-16- Gastric carcinoma	0.0	SJRH30- Rhabdomyosarcoma (met to bone marrow)	2.5
NCI-SNU-1- Gastric carcinoma	26.6	A431- Epidermoid carcinoma	0.0
RF-1- Gastric adenocarcinoma	0.0	WM266-4- Melanoma	0.2
RF-48- Gastric adenocarcinoma	0.1	DU 145- Prostate carcinoma (brain metastasis)	0.0
MKN-45- Gastric carcinoma	0.0	MDA-MB-468- Breast adenocarcinoma	11.2
NCI-N87- Gastric carcinoma	0.0	SCC-4- Squamous cell carcinoma of tongue	0.0
OVCAR-5- Ovarian carcinoma	0.0	SCC-9- Squamous cell carcinoma of tongue	0.0
RL95-2- Uterine carcinoma	0.0	SCC-15- Squamous cell carcinoma of tongue	0.0
HelaS3- Cervical adenocarcinoma	0.0	CAL 27- Squamous cell carcinoma of tongue	0.0

Table 33E. Panel 4D

Tissue Name	Rel. Exp.(%) Ag819, Run 140345750	Rel. Exp.(%) Ag819, Run 145385615	Tissue Name	Rel. Exp.(%) Ag819, Run 140345750	Rel. Exp.(%) Ag819, Run 145385615
Secondary Th1 act	0.0	0.0	HUVEC IL-1beta	0.0	0.0
Secondary Th2 act	0.7	0.2	HUVEC IFN gamma	0.0	0.0
Secondary Tr1 act	0.2	0.3	HUVEC TNF alpha + IFN gamma	0.0	0.0
Secondary Th1 rest	0.0	0.0	HUVEC TNF alpha + IL4	0.0	0.0
Secondary Th2 rest	0.0	0.0	HUVEC IL-11	0.0	0.1
Secondary Tr1 rest	0.0	0.0	Lung Microvascular EC none	0.0	0.0

Primary Th1 act	0.0	0.0	Lung Microvascular EC TNFalpha + IL-1beta	0.0	0.0
Primary Th2 act	0.0	0.0	Microvascular Dermal EC none	0.1	0.0
Primary Tr1 act	0.0	0.0	Microsvasular Dermal EC TNFalpha + IL-1beta	0.0	0.0
Primary Th1 rest	0.0	0.0	Bronchial epithelium TNFalpha + IL1beta	0.0	0.1
Primary Th2 rest	0.0	0.0	Small airway epithelium none	0.0	0.0
Primary Tr1 rest	0.0	0.0	Small airway epithelium TNFalpha + IL-1beta	0.0	0.0
CD45RA CD4 lymphocyte act	0.0	0.1	Coronery artery SMC rest	0.0	0.0
CD45RO CD4 lymphocyte act	0.0	0.1	Coronery artery SMC TNFalpha + IL-1beta	0.0	0.0
CD8 lymphocyte act	0.0	0.0	Astrocytes rest	17.4	12.5
Secondary CD8 lymphocyte rest	0.3	0.1	Astrocytes TNFalpha + IL-1beta	8.5	8.8
Secondary CD8 lymphocyte act	0.0	0.0	KU-812 (Basophil) rest	0.0	0.0
CD4 lymphocyte none	0.0	0.0	KU-812 (Basophil) PMA/ionomycin	0.0	0.0
2ry Th1/Th2/Tr1_anti-CD95 CH11	0.0	0.0	CCD1106 (Keratinocytes) none	0.0	0.0
LAK cells rest	0.0	0.1	CCD1106 (Keratinocytes) TNFalpha + IL-1beta	0.0	0.1
LAK cells IL-2	0.2	0.0	Liver cirrhosis	12.9	10.2
LAK cells IL-2+IL-12	0.0	0.0	Lupus kidney	24.7	34.6
LAK cells IL-2+IFN gamma	0.1	0.3	NCI-H292 none	0.2	0.1

LAK cells IL-2+ IL-18	0.0	0.2	NCI-H292 IL-4	0.1	0.3
LAK cells PMA/ionomycin	0.0	0.0	NCI-H292 IL-9	0.2	0.2
NK Cells IL-2 rest	0.0	0.0	NCI-H292 IL-13	0.2	0.2
Two Way MLR 3 day	0.2	0.3	NCI-H292 IFN gamma	0.0	0.0
Two Way MLR 5 day	0.4	0.2	HPAEC none	0.0	0.1
Two Way MLR 7 day	0.5	0.3	HPAEC TNF alpha + IL-1 beta	0.0	0.0
PBMC rest	0.0	0.0	Lung fibroblast none	0.2	0.0
PBMC PWM	0.8	1.2	Lung fibroblast TNF alpha + IL-1 beta	0.0	0.0
PBMC PHA-L	0.1	0.2	Lung fibroblast IL-4	0.0	0.1
Ramos (B cell) none	0.0	0.1	Lung fibroblast IL-9	0.0	0.0
Ramos (B cell) ionomycin	0.0	0.1	Lung fibroblast IL-13	0.0	0.0
B lymphocytes PWM	0.0	0.0	Lung fibroblast IFN gamma	0.0	0.0
B lymphocytes CD40L and IL-4	0.0	0.0	Dermal fibroblast CCD1070 rest	0.1	0.0
EOL-1 dbcAMP	0.0	0.0	Dermal fibroblast CCD1070 TNF alpha	0.0	0.0
EOL-1 dbcAMP PMA/ionomycin	0.0	0.0	Dermal fibroblast CCD1070 IL-1 beta	0.0	0.0
Dendritic cells none	0.0	0.0	Dermal fibroblast IFN gamma	0.0	0.0
Dendritic cells LPS	0.0	0.0	Dermal fibroblast IL-4	0.0	0.0
Dendritic cells anti-CD40	0.1	0.0	IBD Colitis 2	0.2	0.2
Monocytes rest	0.0	0.0	IBD Crohn's	2.8	1.9
Monocytes LPS	0.0	0.1	Colon	30.8	29.5
Macrophages rest	0.3	0.1	Lung	1.5	1.5
Macrophages LPS	0.0	0.0	Thymus	**100.0**	**100.0**
HUVEC none	0.0	0.0	Kidney	0.4	1.0
HUVEC starved	0.0	0.0			

Table 33F. Panel 5 Islet

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Tissue Name	Rel. Exp.(%) Ag819, Run 229431867	Tissue Name	Rel. Exp.(%) Ag819, Run 229431867
97457_Patient-02go_adipose	0.0	94709_Donor 2 AM - A_adipose	0.0
97476_Patient-07sk_skeletal muscle	0.5	94710_Donor 2 AM - B_adipose	0.0
97477_Patient-07ut_uterus	0.0	94711_Donor 2 AM - C_adipose	0.0
97478_Patient-07pl_placenta	12.4	94712_Donor 2 AD - A_adipose	0.0
99167_Bayer Patient 1	7.9	94713_Donor 2 AD - B_adipose	0.3
97482_Patient-08ut_uterus	0.4	94714_Donor 2 AD - C_adipose	0.0
97483_Patient-08pl_placenta	20.9	94742_Donor 3 U - A_Mesenchymal Stem Cells	0.0
97486_Patient-09sk_skeletal muscle	0.0	94743_Donor 3 U - B_Mesenchymal Stem Cells	0.0
97487_Patient-09ut_uterus	0.0	94730_Donor 3 AM - A_adipose	0.0
97488_Patient-09pl_placenta	11.0	94731_Donor 3 AM - B_adipose	0.0
97492_Patient-10ut_uterus	8.1	94732_Donor 3 AM - C_adipose	0.0
97493_Patient-10pl_placenta	47.0	94733_Donor 3 AD - A_adipose	0.0
97495_Patient-11go_adipose	0.0	94734_Donor 3 AD - B_adipose	0.0
97496_Patient-11sk_skeletal muscle	1.2	94735_Donor 3 AD - C_adipose	0.0
97497_Patient-11ut_uterus	1.5	77138_Liver_HepG2untreated	**100.0**
97498_Patient-11pl_placenta	5.4	73556_Heart_Cardiac stromal cells (primary)	0.0
97500_Patient-12go_adipose	0.0	81735_Small Intestine	38.2
97501_Patient-12sk_skeletal muscle	1.1	72409_Kidney_Proximal Convoluted Tubule	4.9
97502_Patient-12ut_uterus	0.0	82685_Small intestine_Duodenum	1.0
97503_Patient-12pl_placenta	11.7	90650_Adrenal_Adrenocortical adenoma	0.0
94721_Donor 2 U - A_Mesenchymal Stem Cells	0.0	72410_Kidney_HRCE	39.5
94722_Donor 2 U -	0.0	72411_Kidney_HRE	97.9

B_Mesenchymal Stem Cells			
94723_Donor 2 U - C_Mesenchymal Stem Cells	0.0	73139_Uterus_Uterine smooth muscle cells	0.0

Panel 1.2 Summary: Ag819 The expression of the CG55117-01 gene was assessed in two independent runs in panel 1.2 with excellent concordance between the runs. The expression of this gene appears to be highest in a colon cancer cell line (CaCo-2)(CTs=25). In addition, there appears to be substantial expression in colon cancer cell lines and ovarian cancer cell lines. Thus, the expression of this gene could be used to distinguish the CaCo-2 derived sample from other samples in the panel.

Among tissues with metabolic function, this gene has moderate levels of expression in adrenal, thyroid, pituitary, skeletal muscle, and adult and fetal liver. It is highly expressed in pancreas (CT value = 26). Although this gene has no reported dysregulation in metabolic disease, it may be a monoclonal antibody target for the treatment of diseases of the metabolic-endocrine axis, including obesity and Types 1 and 2 diabetes. In addition, this gene appears to be differentially expressed in adult (CT value = 28) vs fetal heart (CT value = 32-33), and may be useful for the identification of the adult phenotype in this tissue.

This gene also shows moderate expression in all CNS regions examined. However, its expression in the fetal brain is considerably higher (greater than 10-fold) suggesting a role in neurodevelopment. Prominin is believed to play a role in the formation of lipid membrane protrusions, their lipid content and membrane to membrane interactions, all critical for synapse formation. The expression of this gene in the developing brain supports a role in synaptogenesis for this molecule. Compensatory synaptogenesis has been shown to occur in the adult brain, especially in response to brain injury or neuronal loss. Therefore, therapeutic modulation of this gene or its protein product may be of therapeutic benefit in clinical conditions where an increase in compensatory synaptogenesis is desirable including stroke, head trauma, spinal cord trauma, Alzheimer's, Parkinson's or Huntington's diseases, multiple sclerosis, or ALS.

References:

Corbeil D, Roper K, Fargeas CA, Joester A, Huttner WB. Prominin: a story of cholesterol, plasma membrane protrusions and human pathology. Traffic 2001 Feb;2(2):82-91

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Prominin is the first identified member of a novel family of polytopic membrane proteins conserved throughout the animal kingdom. It has an unusual membrane topology, containing five transmembrane domains and two large glycosylated extracellular loops. In mammals, prominin is expressed in various embryonic and adult epithelial cells, as well as in

5   nonepithelial cells, such as hematopoietic stem cells. At the subcellular level, prominin is selectively localized in microvilli and other plasma membrane protrusions, irrespective of cell type. At the molecular level, prominin specifically interacts with membrane cholesterol and is a marker of a novel type of cholesterol-based lipid 'raft'. A frameshift mutation in the human prominin gene, which results in a truncated protein that is no longer transported to the cell

10   surface, is associated with retinal degeneration. Given that prominin is concentrated in the plasma membrane evaginations at the base of the outer segment of rod photoreceptor cells, which are essential precursor structures in the biogenesis of photoreceptive disks, it is proposed that prominin has a role in the generation of plasma membrane protrusions, their lipid composition and organization and their membrane-to-membrane interactions.

15   **Panel 2D Summary:** Ag819 The expression of the CG55117-01 gene was assessed in two independent runs in panel 2D with the runs showing excellent concordance. The expression of this gene is found to be highest in samples derived from an ovarian cancer and a lung cancer (CTs=26). In addition, there is substantial expression seen in other cancer samples including a kidney cancer, two colon cancers and two gastric cancers. Thus, the expression of this gene

20   could be used to distinguish the ovarian or lung cancer from the other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be of benefit in the treatment of ovarian, lung, kidney, colon or gastric cancers.

Panel 3D Summary: Ag819 The expression of the CG55117-01 gene is highest in a sample

25   derived from a brain cancer (medulloblastoma) derived cell line (D283 cells)(CT=28.3). In addition, there is substantial expression seen in several lung cancer cell lines, a gastric cancer cell line and several pancreatic cancer cell lines. Thus, the expression of this gene could be used to distinguish D283 cells from other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein

30   therapeutics might be of benefit in the treatment of lung, brain, pancreatic or gastric cancers.

Panel 4D Summary: Ag819 The CG55117-01 gene, which encodes a 5-transmembrane protein, human haematopoietic progenitor cell antigen AC133 homolog is expressed at moderate levels in thymus, colon, and lupus kidney. Therefore, antibodies and small molecule

drugs that antagonize the function of the CG55117-01 gene product may reduce or eliminate the symptoms in patients with lupus nephritis.

Panel 5 Islet Summary: Ag819 The CG55117-01 gene has low levels of expression in placenta and islets (CTs=32-34). Thus, expression of this gene could be used to differentiate samples derived from these tissues from other samples on this panel.

NOV6 (CG55690-01)

Expression of gene CG55690-01 was assessed using the primer-probe sets Ag2256 and Ag4933, described in Tables 34A and 34B. Results of the RTQ-PCR runs are shown in Tables 34C, 34D, 34E, 34F, 34G, 34H, and 34I.

Table 34A. Probe Name Ag2256

Primers	Sequences	Length	Start Position
Forward	5'-cacgcactgccactataagg-3' (SEQ ID NO:195)	20	1717
Probe	TET-5'-cttgcacatgactaagacggacccct-3'-TAMRA (SEQ ID NO:196)	26	1750
Reverse	5'-ctagaggtgtgtggggttctc-3' (SEQ ID NO:197)	21	1781

Table 34B. Probe Name Ag4933

Primers	Sequences	Length	Start Position
Forward	5'-cacgcactgccactataagg-3' (SEQ ID NO:198)	20	1717
Probe	TET-5'-cttgcacatgactaagacggacccct-3'-TAMRA (SEQ ID NO:199)	26	1750
Reverse	5'-ctagaggtgtgtggggttctc-3' (SEQ ID NO:200)	21	1781

Table 34C. CNS_neurodegeneration_v1.0

Tissue Name	Rel. Exp.(%) Ag2256, Run 207967145	Tissue Name	Rel. Exp.(%) Ag2256, Run 207967145
AD 1 Hippo	83.5	Control (Path) 3 Temporal Ctx	57.4
AD 2 Hippo	75.3	Control (Path) 4 Temporal Ctx	31.4
AD 3 Hippo	19.5	AD 1 Occipital Ctx	33.7
AD 4 Hippo	13.1	AD 2 Occipital Ctx (Missing)	1.4
AD 5 hippo	92.0	AD 3 Occipital Ctx	57.8
AD 6 Hippo	57.8	AD 4 Occipital Ctx	27.0
Control 2 Hippo	49.7	AD 5 Occipital Ctx	15.2
Control 4 Hippo	12.3	AD 6 Occipital Ctx	29.3
Control (Path) 3 Hippo	28.1	Control 1 Occipital Ctx	12.7
AD 1 Temporal Ctx	47.6	Control 2 Occipital Ctx	41.8
AD 2 Temporal Ctx	37.1	Control 3 Occipital	27.2

		Ctx	
AD 3 Temporal Ctx	49.0	Control 4 Occipital Ctx	27.7
AD 4 Temporal Ctx	80.1	Control (Path) 1 Occipital Ctx	40.9
AD 5 Inf Temporal Ctx	58.2	Control (Path) 2 Occipital Ctx	11.0
AD 5 SupTemporal Ctx	34.9	Control (Path) 3 Occipital Ctx	19.3
AD 6 Inf Temporal Ctx	28.9	Control (Path) 4 Occipital Ctx	22.5
AD 6 Sup Temporal Ctx	31.0	Control 1 Parietal Ctx	1.5
Control 1 Temporal Ctx	7.1	Control 2 Parietal Ctx	61.6
Control 2 Temporal Ctx	31.6	Control 3 Parietal Ctx	12.1
Control 3 Temporal Ctx	59.0	Control (Path) 1 Parietal Ctx	20.7
Control 4 Temporal Ctx	44.8	Control (Path) 2 Parietal Ctx	50.0
Control (Path) 1 Temporal Ctx	**100.0**	Control (Path) 3 Parietal Ctx	52.1
Control (Path) 2 Temporal Ctx	29.3	Control (Path) 4 Parietal Ctx	15.8

Table 34D. General_screening_panel_v1.5

Tissue Name	Rel. Exp.(%) Ag2256, Run 229393821	Rel. Exp.(%) Ag4933, Run 228843452	Tissue Name	Rel. Exp.(%) Ag2256, Run 229393821	Rel. Exp.(%) Ag4933, Run 228843452
Adipose	8.1	4.3	Renal ca. TK-10	4.1	6.5
Melanoma* Hs688(A).T	4.1	4.3	Bladder	2.6	2.0
Melanoma* Hs688(B).T	1.0	2.8	Gastric ca. (liver met.) NCI-N87	0.3	0.0
Melanoma* M14	19.6	27.0	Gastric ca. KATO III	0.0	0.0
Melanoma* LOXIMVI	0.9	0.0	Colon ca. SW-948	5.2	1.0
Melanoma* SK-MEL-5	30.6	24.7	Colon ca. SW480	48.0	79.0
Squamous cell carcinoma SCC-4	0.4	0.0	Colon ca.* (SW480 met) SW620	31.4	31.6
Testis Pool	6.2	9.6	Colon ca. HT29	0.0	1.4
Prostate ca.*	3.6	3.3	Colon ca. HCT-	7.6	7.5

(bone met) PC-3			116		
Prostate Pool	3.6	2.6	Colon ca. CaCo-2	2.3	9.7
Placenta	5.3	2.2	Colon cancer tissue	0.8	2.2
Uterus Pool	0.2	0.0	Colon ca. SW1116	0.0	0.0
Ovarian ca. OVCAR-3	2.5	5.4	Colon ca. Colo-205	0.0	0.0
Ovarian ca. SK-OV-3	17.2	13.6	Colon ca. SW-48	0.0	1.1
Ovarian ca. OVCAR-4	11.3	12.9	Colon Pool	3.4	5.3
Ovarian ca. OVCAR-5	15.0	10.2	Small Intestine Pool	2.7	1.0
Ovarian ca. IGROV-1	3.2	12.1	Stomach Pool	0.0	1.0
Ovarian ca. OVCAR-8	25.9	36.9	Bone Marrow Pool	0.8	1.9
Ovary	1.1	0.3	Fetal Heart	0.0	0.0
Breast ca. MCF-7	4.0	1.9	Heart Pool	0.0	0.2
Breast ca. MDA-MB-231	0.9	1.0	Lymph Node Pool	4.2	2.1
Breast ca. BT 549	7.7	17.4	Fetal Skeletal Muscle	6.2	8.9
Breast ca. T47D	3.8	6.9	Skeletal Muscle Pool	23.2	30.1
Breast ca. MDA-N	0.0	0.0	Spleen Pool	0.8	1.1
Breast Pool	0.7	0.0	Thymus Pool	2.1	0.9
Trachea	14.9	15.8	CNS cancer (glio/astro) U87-MG	0.0	0.0
Lung	0.0	0.0	CNS cancer (glio/astro) U-118-MG	0.0	0.0
Fetal Lung	3.5	4.6	CNS cancer (neuro;met) SK-N-AS	0.7	0.0
Lung ca. NCI-N417	17.9	41.8	CNS cancer (astro) SF-539	2.6	7.0
Lung ca. LX-1	4.6	6.0	CNS cancer (astro) SNB-75	15.5	16.8
Lung ca. NCI-	**100.0**	**100.0**	CNS cancer	13.5	16.5

H146			(glio) SNB-19		
Lung ca. SHP-77	2.3	3.0	CNS cancer (glio) SF-295	0.0	0.0
Lung ca. A549	0.0	4.6	Brain (Amygdala) Pool	11.4	13.3
Lung ca. NCI-H526	6.0	2.5	Brain (cerebellum)	21.0	19.5
Lung ca. NCI-H23	26.8	35.4	Brain (fetal)	11.9	18.4
Lung ca. NCI-H460	27.9	29.9	Brain (Hippocampus) Pool	7.3	7.1
Lung ca. HOP-62	0.0	3.0	Cerebral Cortex Pool	6.2	7.6
Lung ca. NCI-H522	16.7	17.4	Brain (Substantia nigra) Pool	13.7	11.0
Liver	0.1	2.4	Brain (Thalamus) Pool	9.8	11.7
Fetal Liver	6.2	4.6	Brain (whole)	8.5	17.7
Liver ca. HepG2	4.7	8.2	Spinal Cord Pool	17.1	15.3
Kidney Pool	1.8	4.2	Adrenal Gland	0.7	2.6
Fetal Kidney	2.0	0.0	Pituitary gland Pool	3.1	8.0
Renal ca. 786-0	0.0	0.0	Salivary Gland	1.6	8.0
Renal ca. A498	4.6	6.7	Thyroid (female)	1.6	2.7
Renal ca. ACHN	1.1	3.5	Pancreatic ca. CAPAN2	0.0	0.0
Renal ca. UO-31	2.2	7.6	Pancreas Pool	2.1	4.2

Table 34E. Panel 1.3D

Tissue Name	Rel. Exp.(%) Ag2256, Run 148422104	Rel. Exp.(%) Ag2256, Run 148493664	Tissue Name	Rel. Exp.(%) Ag2256, Run 148422104	Rel. Exp.(%) Ag2256, Run 148493664
Liver adenocarcinoma	0.0	0.0	Kidney (fetal)	1.3	1.8
Pancreas	0.0	0.9	Renal ca. 786-0	1.0	0.0
Pancreatic ca. CAPAN 2	0.0	0.0	Renal ca. A498	0.5	4.7
Adrenal gland	3.2	4.1	Renal ca. RXF 393	0.0	0.0
Thyroid	0.0	0.0	Renal ca.	1.4	2.3

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			ACHN		
Salivary gland	7.9	5.5	Renal ca. UO-31	0.0	6.1
Pituitary gland	12.2	1.7	Renal ca. TK-10	0.0	0.0
Brain (fetal)	1.3	1.6	Liver	0.0	0.0
Brain (whole)	11.7	20.2	Liver (fetal)	1.2	12.9
Brain (amygdala)	18.8	31.0	Liver ca. (hepatoblast) HepG2	2.1	0.0
Brain (cerebellum)	0.0	0.0	Lung	1.2	0.0
Brain (hippocampus)	85.9	95.3	Lung (fetal)	2.8	2.2
Brain (substantia nigra)	8.9	10.9	Lung ca. (small cell) LX-1	0.0	4.4
Brain (thalamus)	70.7	39.5	Lung ca. (small cell) NCI-H69	23.5	30.8
Cerebral Cortex	17.1	11.9	Lung ca. (s.cell var.) SHP-77	0.2	3.8
Spinal cord	2.7	4.0	Lung ca. (large cell)NCI-H460	8.5	2.9
glio/astro U87-MG	0.0	0.9	Lung ca. (non-sm. cell) A549	1.6	2.2
glio/astro U-118-MG	0.0	0.0	Lung ca. (non-s.cell) NCI-H23	8.8	13.6
astrocytoma SW1783	12.4	12.4	Lung ca. (non-s.cell) HOP-62	0.0	1.7
neuro*; met SK-N-AS	0.0	0.0	Lung ca. (non-s.cl) NCI-H522	6.0	10.1
astrocytoma SF-539	1.6	7.0	Lung ca. (squam.) SW 900	1.0	4.7
astrocytoma SNB-75	0.0	4.5	Lung ca. (squam.) NCI-H596	31.9	27.9
glioma SNB-19	0.0	0.0	Mammary gland	0.0	5.1
glioma U251	0.0	0.0	Breast ca.* (pl.ef) MCF-7	0.0	0.0
glioma SF-295	0.0	0.0	Breast ca.* (pl.ef) MDA-MB-231	0.0	0.0

Heart (fetal)	1.1	2.1	Breast ca.* (pl.ef) T47D	2.5	3.4
Heart	0.0	0.0	Breast ca. BT-549	7.3	6.3
Skeletal muscle (fetal)	**100.0**	**100.0**	Breast ca. MDA-N	0.0	0.0
Skeletal muscle	1.1	11.7	Ovary	2.1	4.5
Bone marrow	2.9	0.0	Ovarian ca. OVCAR-3	0.0	2.0
Thymus	0.0	2.3	Ovarian ca. OVCAR-4	1.3	2.0
Spleen	0.6	0.0	Ovarian ca. OVCAR-5	0.0	0.0
Lymph node	1.3	3.4	Ovarian ca. OVCAR-8	3.2	0.0
Colorectal	5.4	0.0	Ovarian ca. IGROV-1	0.0	0.0
Stomach	0.6	2.9	Ovarian ca.* (ascites) SK-OV-3	1.5	2.0
Small intestine	15.6	11.7	Uterus	1.1	1.1
Colon ca. SW480	15.9	33.2	Placenta	2.1	2.5
Colon ca.* SW620(SW480 met)	1.3	0.0	Prostate	5.7	11.6
Colon ca. HT29	0.0	0.0	Prostate ca.* (bone met)PC-3	1.1	0.0
Colon ca. HCT-116	0.0	0.0	Testis	40.9	85.3
Colon ca. CaCo-2	1.2	1.3	Melanoma Hs688(A).T	2.3	0.0
Colon ca. tissue(ODO3866)	0.0	0.0	Melanoma* (met) Hs688(B).T	1.3	5.7
Colon ca. HCC-2998	8.9	13.6	Melanoma UACC-62	6.8	2.2
Gastric ca.* (liver met) NCI-N87	0.0	2.3	Melanoma M14	7.7	14.6
Bladder	0.0	2.6	Melanoma LOX IMVI	0.0	0.0
Trachea	18.4	15.5	Melanoma* (met) SK-MEL-5	3.7	9.2
Kidney	0.0	1.0	Adipose	2.4	2.3

Table 34F. Panel 2D

Tissue Name	Rel. Exp.(%) Ag2256, Run 148422111	Rel. Exp.(%) Ag2256, Run 148493675	Tissue Name	Rel. Exp.(%) Ag2256, Run 148422111	Rel. Exp.(%) Ag2256, Run 148493675
Normal Colon	3.3	5.0	Kidney Margin 8120608	6.7	0.0
CC Well to Mod Diff (ODO3866)	5.7	1.0	Kidney Cancer 8120613	0.0	0.0
CC Margin (ODO3866)	15.4	5.8	Kidney Margin 8120614	0.0	0.0
CC Gr.2 rectosigmoid (ODO3868)	0.0	0.0	Kidney Cancer 9010320	6.2	0.0
CC Margin (ODO3868)	0.0	4.7	Kidney Margin 9010321	3.1	2.5
CC Mod Diff (ODO3920)	1.6	11.1	Normal Uterus	6.3	0.0
CC Margin (ODO3920)	0.0	2.5	Uterus Cancer 064011	0.0	0.0
CC Gr.2 ascend colon (ODO3921)	16.7	2.2	Normal Thyroid	8.3	4.9
CC Margin (ODO3921)	5.6	4.3	Thyroid Cancer 064010	2.9	0.0
CC from Partial Hepatectomy (ODO4309) Mets	2.5	0.0	Thyroid Cancer A302152	0.0	0.0
Liver Margin (ODO4309)	7.7	0.0	Thyroid Margin A302153	6.1	10.3
Colon mets to lung (OD04451-01)	39.2	31.6	Normal Breast	12.9	5.9
Lung Margin (OD04451-02)	0.0	0.0	Breast Cancer (OD04566)	2.6	3.9
Normal Prostate 6546-1	21.9	19.3	Breast Cancer (OD04590-01)	0.0	1.9
Prostate Cancer (OD04410)	10.6	14.9	Breast Cancer Mets (OD04590-03)	6.0	13.5
Prostate Margin (OD04410)	48.3	30.1	Breast Cancer Metastasis (OD04655-05)	8.4	0.0

Prostate Cancer (OD04720-01)	14.1	5.0	Breast Cancer 064006	0.0	5.0
Prostate Margin (OD04720-02)	21.5	20.7	Breast Cancer 1024	3.2	6.5
Normal Lung 061010	6.8	9.0	Breast Cancer 9100266	0.0	0.0
Lung Met to Muscle (ODO4286)	0.0	1.1	Breast Margin 9100265	3.1	0.0
Muscle Margin (ODO4286)	24.0	9.3	Breast Cancer A209073	3.4	3.2
Lung Malignant Cancer (OD03126)	5.4	0.0	Breast Margin A2090734	6.7	6.0
Lung Margin (OD03126)	9.7	4.9	Normal Liver	0.0	0.0
Lung Cancer (OD04404)	13.7	11.7	Liver Cancer 064003	5.1	0.0
Lung Margin (OD04404)	3.0	2.4	Liver Cancer 1025	0.0	0.0
Lung Cancer (OD04565)	0.0	0.0	Liver Cancer 1026	5.0	25.9
Lung Margin (OD04565)	0.0	0.0	Liver Cancer 6004-T	0.0	0.0
Lung Cancer (OD04237-01)	**100.0**	**100.0**	Liver Tissue 6004-N	5.0	11.8
Lung Margin (OD04237-02)	0.0	0.0	Liver Cancer 6005-T	16.2	18.4
Ocular Mel Met to Liver (ODO4310)	77.9	73.2	Liver Tissue 6005-N	0.0	0.0
Liver Margin (ODO4310)	0.0	3.0	Normal Bladder	4.5	2.7
Melanoma Mets to Lung (OD04321)	39.5	39.8	Bladder Cancer 1023	0.0	1.1
Lung Margin (OD04321)	0.0	4.9	Bladder Cancer A302173	7.3	11.0
Normal Kidney	6.0	4.1	Bladder Cancer (OD04718-01)	3.7	0.0
Kidney Ca, Nuclear grade 2 (OD04338)	2.9	12.9	Bladder Normal Adjacent (OD04718-03)	1.4	5.4
Kidney Margin	3.7	0.0	Normal Ovary	4.0	8.0

(OD04338)					
Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	0.0	Ovarian Cancer 064008	8.1	2.7
Kidney Margin (OD04339)	2.3	0.0	Ovarian Cancer (OD04768-07)	13.7	11.4
Kidney Ca, Clear cell type (OD04340)	2.5	4.0	Ovary Margin (OD04768-08)	1.6	2.0
Kidney Margin (OD04340)	9.3	4.2	Normal Stomach	7.6	1.5
Kidney Ca, Nuclear grade 3 (OD04348)	0.0	0.0	Gastric Cancer 9060358	1.7	0.0
Kidney Margin (OD04348)	2.9	0.0	Stomach Margin 9060359	5.6	0.0
Kidney Cancer (OD04622-01)	11.3	2.3	Gastric Cancer 9060395	0.0	2.5
Kidney Margin (OD04622-03)	0.0	0.0	Stomach Margin 9060394	3.5	2.7
Kidney Cancer (OD04450-01)	12.7	18.6	Gastric Cancer 9060397	0.0	0.0
Kidney Margin (OD04450-03)	0.0	0.0	Stomach Margin 9060396	0.0	0.0
Kidney Cancer 8120607	3.1	4.8	Gastric Cancer 064005	0.0	0.0

Table 34G. Panel 3D

Tissue Name	Rel. Exp.(%) Ag2256, Run 170745682	Tissue Name	Rel. Exp.(%) Ag2256, Run 170745682
Daoy- Medulloblastoma	0.5	Ca Ski- Cervical epidermoid carcinoma (metastasis)	12.8
TE671- Medulloblastoma	5.3	ES-2- Ovarian clear cell carcinoma	1.0
D283 Med- Medulloblastoma	7.0	Ramos- Stimulated with PMA/ionomycin 6h	0.0
PFSK-1- Primitive Neuroectodermal	1.5	Ramos- Stimulated with PMA/ionomycin 14h	0.0
XF-498- CNS	0.0	MEG-01- Chronic myelogenous leukemia (megokaryoblast)	0.0
SNB-78- Glioma	0.0	Raji- Burkitt's lymphoma	0.0

SF-268- Glioblastoma	2.0	Daudi- Burkitt's lymphoma	0.0
T98G- Glioblastoma	0.0	U266- B-cell plasmacytoma	0.2
SK-N-SH- Neuroblastoma (metastasis)	15.8	CA46- Burkitt's lymphoma	0.0
SF-295- Glioblastoma	0.0	RL- non-Hodgkin's B-cell lymphoma	0.0
Cerebellum	6.4	JM1- pre-B-cell lymphoma	0.0
Cerebellum	6.5	Jurkat- T cell leukemia	0.0
NCI-H292- Mucoepidermoid lung carcinoma	0.0	TF-1- Erythroleukemia	0.7
DMS-114- Small cell lung cancer	22.2	HUT 78- T-cell lymphoma	0.0
DMS-79- Small cell lung cancer	0.0	U937- Histiocytic lymphoma	3.6
NCI-H146- Small cell lung cancer	100.0	KU-812- Myelogenous leukemia	0.0
NCI-H526- Small cell lung cancer	9.5	769-P- Clear cell renal carcinoma	0.0
NCI-N417- Small cell lung cancer	26.1	Caki-2- Clear cell renal carcinoma	0.9
NCI-H82- Small cell lung cancer	1.7	SW 839- Clear cell renal carcinoma	0.0
NCI-H157- Squamous cell lung cancer (metastasis)	0.0	G401- Wilms' tumor	0.0
NCI-H1155- Large cell lung cancer	24.5	Hs766T- Pancreatic carcinoma (LN metastasis)	0.0
NCI-H1299- Large cell lung cancer	14.9	CAPAN-1- Pancreatic adenocarcinoma (liver metastasis)	0.0
NCI-H727- Lung carcinoid	1.8	SU86.86- Pancreatic carcinoma (liver metastasis)	0.1
NCI-UMC-11- Lung carcinoid	1.7	BxPC-3- Pancreatic adenocarcinoma	1.5
LX-1- Small cell lung cancer	6.3	HPAC- Pancreatic adenocarcinoma	0.0
Colo-205- Colon cancer	0.0	MIA PaCa-2- Pancreatic carcinoma	1.4
KM12- Colon cancer	4.4	CFPAC-1- Pancreatic ductal adenocarcinoma	1.4
KM20L2- Colon cancer	0.0	PANC-1- Pancreatic epithelioid ductal carcinoma	4.6
NCI-H716- Colon cancer	7.2	T24- Bladder carcinma (transitional cell)	0.0

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SW-48- Colon adenocarcinoma	0.0	5637- Bladder carcinoma	0.0
SW1116- Colon adenocarcinoma	0.0	HT-1197- Bladder carcinoma	0.0
LS 174T- Colon adenocarcinoma	0.0	UM-UC-3- Bladder carcinma (transitional cell)	0.0
SW-948- Colon adenocarcinoma	0.0	A204- Rhabdomyosarcoma	3.5
SW-480- Colon adenocarcinoma	0.0	HT-1080- Fibrosarcoma	0.0
NCI-SNU-5- Gastric carcinoma	6.7	MG-63- Osteosarcoma	0.0
KATO III- Gastric carcinoma	23.2	SK-LMS-1- Leiomyosarcoma (vulva)	0.0
NCI-SNU-16- Gastric carcinoma	0.0	SJRH30- Rhabdomyosarcoma (met to bone marrow)	0.0
NCI-SNU-1- Gastric carcinoma	0.0	A431- Epidermoid carcinoma	0.0
RF-1- Gastric adenocarcinoma	0.9	WM266-4- Melanoma	4.9
RF-48- Gastric adenocarcinoma	0.0	DU 145- Prostate carcinoma (brain metastasis)	0.0
MKN-45- Gastric carcinoma	0.0	MDA-MB-468- Breast adenocarcinoma	4.1
NCI-N87- Gastric carcinoma	0.0	SCC-4- Squamous cell carcinoma of tongue	0.0
OVCAR-5- Ovarian carcinoma	0.3	SCC-9- Squamous cell carcinoma of tongue	0.0
RL95-2- Uterine carcinoma	2.0	SCC-15- Squamous cell carcinoma of tongue	0.0
HelaS3- Cervical adenocarcinoma	0.0	CAL 27- Squamous cell carcinoma of tongue	0.0

Table 34H. Panel 4.1D

Tissue Name	Rel. Exp.(%) Ag4933, Run 223597253	Tissue Name	Rel. Exp.(%) Ag4933, Run 223597253
Secondary Th1 act	1.5	HUVEC IL-1beta	0.4
Secondary Th2 act	1.5	HUVEC IFN gamma	1.6
Secondary Tr1 act	2.5	HUVEC TNF alpha + IFN gamma	1.9
Secondary Th1 rest	0.0	HUVEC TNF alpha + IL4	0.0
Secondary Th2 rest	0.0	HUVEC IL-11	1.0
Secondary Tr1 rest	0.0	Lung Microvascular EC none	13.4
Primary Th1 act	7.1	Lung Microvascular EC	3.4

		TNFalpha + IL-1beta	
Primary Th2 act	7.1	Microvascular Dermal EC none	0.7
Primary Tr1 act	3.5	Microsvasular Dermal EC TNFalpha + IL-1beta	4.8
Primary Th1 rest	0.8	Bronchial epithelium TNFalpha + IL1beta	0.0
Primary Th2 rest	0.0	Small airway epithelium none	0.0
Primary Tr1 rest	0.0	Small airway epithelium TNFalpha + IL-1beta	0.0
CD45RA CD4 lymphocyte act	0.0	Coronery artery SMC rest	0.0
CD45RO CD4 lymphocyte act	0.0	Coronery artery SMC TNFalpha + IL-1beta	0.0
CD8 lymphocyte act	0.0	Astrocytes rest	0.0
Secondary CD8 lymphocyte rest	1.4	Astrocytes TNFalpha + IL-1beta	2.7
Secondary CD8 lymphocyte act	2.3	KU-812 (Basophil) rest	0.0
CD4 lymphocyte none	0.0	KU-812 (Basophil) PMA/ionomycin	0.9
2ry Th1/Th2/Tr1_anti-CD95 CH11	0.0	CCD1106 (Keratinocytes) none	0.0
LAK cells rest	1.3	CCD1106 (Keratinocytes) TNFalpha + IL-1beta	4.6
LAK cells IL-2	0.0	Liver cirrhosis	0.5
LAK cells IL-2+IL-12	0.0	NCI-H292 none	1.5
LAK cells IL-2+IFN gamma	0.0	NCI-H292 IL-4	0.0
LAK cells IL-2+ IL-18	0.0	NCI-H292 IL-9	0.0
LAK cells PMA/ionomycin	0.0	NCI-H292 IL-13	0.0
NK Cells IL-2 rest	0.0	NCI-H292 IFN gamma	0.0
Two Way MLR 3 day	0.0	HPAEC none	0.0
Two Way MLR 5 day	0.0	HPAEC TNF alpha + IL-1 beta	0.0
Two Way MLR 7 day	1.9	Lung fibroblast none	0.7
PBMC rest	0.0	Lung fibroblast TNF alpha + IL-1 beta	1.5
PBMC PWM	1.5	Lung fibroblast IL-4	3.3
PBMC PHA-L	0.0	Lung fibroblast IL-9	4.4
Ramos (B cell) none	0.0	Lung fibroblast IL-13	4.2
Ramos (B cell) ionomycin	0.0	Lung fibroblast IFN gamma	2.6

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B lymphocytes PWM	1.6	Dermal fibroblast CCD1070 rest	1.7
B lymphocytes CD40L and IL-4	0.9	Dermal fibroblast CCD1070 TNF alpha	0.0
EOL-1 dbcAMP	0.0	Dermal fibroblast CCD1070 IL-1 beta	3.6
EOL-1 dbcAMP PMA/ionomycin	0.0	Dermal fibroblast IFN gamma	0.0
Dendritic cells none	0.0	Dermal fibroblast IL-4	3.8
Dendritic cells LPS	0.0	Dermal Fibroblasts rest	3.4
Dendritic cells anti-CD40	0.0	Neutrophils TNFa+LPS	4.8
Monocytes rest	0.0	Neutrophils rest	3.4
Monocytes LPS	0.0	Colon	3.8
Macrophages rest	0.0	Lung	6.3
Macrophages LPS	1.6	Thymus	38.4
HUVEC none	0.0	Kidney	**100.0**
HUVEC starved	1.7		

Table 34I. Panel 4D

Tissue Name	Rel. Exp.(%) Ag2256, Run 148493657	Tissue Name	Rel. Exp.(%) Ag2256, Run 148493657
Secondary Th1 act	1.6	HUVEC IL-1beta	3.6
Secondary Th2 act	0.0	HUVEC IFN gamma	0.0
Secondary Tr1 act	7.7	HUVEC TNF alpha + IFN gamma	0.0
Secondary Th1 rest	0.0	HUVEC TNF alpha + IL4	0.0
Secondary Th2 rest	0.0	HUVEC IL-11	4.1
Secondary Tr1 rest	0.0	Lung Microvascular EC none	12.9
Primary Th1 act	11.1	Lung Microvascular EC TNFalpha + IL-1beta	5.5
Primary Th2 act	17.0	Microvascular Dermal EC none	0.0
Primary Tr1 act	7.9	Microsvasular Dermal EC TNFalpha + IL-1beta	23.5
Primary Th1 rest	4.7	Bronchial epithelium TNFalpha + IL1beta	0.0
Primary Th2 rest	0.0	Small airway epithelium none	0.0
Primary Tr1 rest	3.9	Small airway epithelium TNFalpha + IL-1beta	3.8
CD45RA CD4 lymphocyte act	5.6	Coronery artery SMC rest	0.0

CD45RO CD4 lymphocyte act	3.8	Coronery artery SMC TNFalpha + IL-1beta	3.4
CD8 lymphocyte act	0.0	Astrocytes rest	0.0
Secondary CD8 lymphocyte rest	0.0	Astrocytes TNFalpha + IL-1beta	20.7
Secondary CD8 lymphocyte act	2.3	KU-812 (Basophil) rest	0.0
CD4 lymphocyte none	0.0	KU-812 (Basophil) PMA/ionomycin	0.0
2ry Th1/Th2/Tr1_anti-CD95 CH11	3.8	CCD1106 (Keratinocytes) none	8.2
LAK cells rest	0.0	CCD1106 (Keratinocytes) TNFalpha + IL-1beta	16.5
LAK cells IL-2	3.9	Liver cirrhosis	36.6
LAK cells IL-2+IL-12	0.0	Lupus kidney	0.0
LAK cells IL-2+IFN gamma	0.0	NCI-H292 none	0.0
LAK cells IL-2+ IL-18	1.6	NCI-H292 IL-4	0.0
LAK cells PMA/ionomycin	9.4	NCI-H292 IL-9	4.0
NK Cells IL-2 rest	0.0	NCI-H292 IL-13	4.5
Two Way MLR 3 day	0.0	NCI-H292 IFN gamma	0.0
Two Way MLR 5 day	3.1	HPAEC none	4.8
Two Way MLR 7 day	7.8	HPAEC TNF alpha + IL-1 beta	3.4
PBMC rest	0.0	Lung fibroblast none	0.0
PBMC PWM	3.2	Lung fibroblast TNF alpha + IL-1 beta	0.0
PBMC PHA-L	0.0	Lung fibroblast IL-4	2.6
Ramos (B cell) none	0.0	Lung fibroblast IL-9	9.3
Ramos (B cell) ionomycin	0.0	Lung fibroblast IL-13	11.8
B lymphocytes PWM	20.9	Lung fibroblast IFN gamma	7.1
B lymphocytes CD40L and IL-4	0.0	Dermal fibroblast CCD1070 rest	0.0
EOL-1 dbcAMP	0.0	Dermal fibroblast CCD1070 TNF alpha	0.0
EOL-1 dbcAMP PMA/ionomycin	0.0	Dermal fibroblast CCD1070 IL-1 beta	27.4
Dendritic cells none	4.1	Dermal fibroblast IFN gamma	0.0
Dendritic cells LPS	0.0	Dermal fibroblast IL-4	3.0
Dendritic cells anti-CD40	0.0	IBD Colitis 2	4.0

Monocytes rest	0.0	IBD Crohn's	3.6
Monocytes LPS	0.0	Colon	**100.0**
Macrophages rest	0.0	Lung	45.1
Macrophages LPS	2.4	Thymus	0.0
HUVEC none	0.0	Kidney	3.6
HUVEC starved	0.0		

CNS_neurodegeneration_v1.0 Summary: Ag2256 Expression of the CG55690-01 gene is not differentially expressed in Alzheimer's disease, based on the expression in this panel. However, these results confirm expression of this gene in the brain. Please see General_screening_panel_v1.5 for discussion of utility of this gene in the central nervous system.

General_screening_panel_v1.5 Summary: Ag2256/Ag4933 Two experiments with the same probe and primer set show highest expression of the CG55690-01 gene in a sample derived from a lung cancer cell line (NCI-H146) (CTs=30). In addition, there are a number of lung cancer cell lines expressing this gene as well as colon cancer and ovarian cancer cell lines. Thus, the expression of this gene could be used to distinguish NCI-H146 cells from other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, protein therapeutics or antibodies might be of benefit in the treatment of lung cancer, colon cancer or ovarian cancer.

This gene also has moderate expression in adipose, adult and fetal skeletal muscle, and pituitary. Although this gene product has no reported associations with metabolic disease/metabolism, its expression profile suggests that it may be a monoclonal antibody target for the treatment of metabolic and endocrine disease, including obesity and Types 1 and 2 diabetes.

In addition, this gene is expressed at low levels in all CNS regions examined. This gene is a homolog of Frizzled. Frizzled genes play a role in cell fate determination. Therefore, this gene may be of use in stem cell research and therapy, specifically to control the differentiation of stem cells into post-mitotic neurons.

References:

Moriwaki J, Kajita E, Kirikoshi H, Koike J, Sagara N, Yasuhiko Y, Saitoh T, Hirai M, Katoh M, Shiokawa K. Isolation of Xenopus frizzled-10A and frizzled-10B genomic clones and their expression in adult tissues and embryos. Biochem Biophys Res Commun 2000 Nov 19;278(2):377-84

Frizzled genes, encoding WNT receptors, play key roles in cell fate determination. Here, we isolated two Xenopus frizzled genes (Xfz10A and Xfz10B), probably reflecting pseudotetraploidy in Xenopus. Xfz10A (586 amino acids) and Xfz10B (580 amino acids) both encoded by a single exon, consisted of the N-terminal cysteine-rich domain, seven

5   transmembrane domains, and the C-terminal Ser/Thr-X-Val motif. Xfz10A and Xfz10B were 97.0% identical at the amino acid level, and Xfz10B was 100% identical to previously reported Xfz9, yet Xfz10A was 85.3% and 62.4% identical to FZD10 and FZD9, respectively. Xfz10 mRNA appeared as 3.4 kb in adult tissues and embryos. RT-PCR analyses revealed the expression of more Xfz10A mRNA in stomach, kidney, eye, skeletal muscle, and skin, and

10   more Xfz10B mRNA in heart and ovary, but in embryos, two mRNAs were equally expressed from the blastula stage with their peak expression at the late gastrula stage. The main site of Xfz10 mRNA expression was neural fold at the neurula stage and the dorsal region of midbrain, hindbrain, and spinal cord at the tadpole stage. These results suggest that Xfz10 has important roles in neural tissue formation.

15   **Panel 1.3D Summary:** Ag2256 Two experiments with the same probe and primer set show highest expression of the CG55690-01 gene in fetal skeletal muscle (CTs=31-33). This gene is expressed at much higher levels in fetal skeletal muscle than in adult skeletal muscle (CTs=36-37). Therefore, expression of this gene could be used to differentiate between adult and fetal skeletal muscle. In addition, the higher levels of expression in fetal skeletal muscle suggest

20   that the protein product may enhance muscular growth or development in the fetus and thus may also act in a regenerative capacity in the adult. Therefore, therapeutic modulation of the protein encoded by this gene could be useful in treatment of muscle related diseases. More specifically, treatment of weak or dystrophic muscle with the protein encoded by this gene could restore muscle mass or function.

25   This panel also shows expression of this gene in the CNS. Please see General Screening Panel 1.5 for a discussion of utility of this gene in the central nervous system.

Panel 2D Summary: Ag2256 The expression of the CG55690-01 gene was assessed in two independent runs in panel 2D with excellent concordance between runs. The expression of this gene is found to be highest in a sample derived from a lung cancer (CTs=30). In addition,

30   other lung cancers were found to express this gene, while their normal adjacent tissue counterparts were low to undetectable for the expression of this gene. This expression is consistent with the expression seen in General_screening_panel_v1.5. Thus, the expression of this gene could be used to distinguish lung cancer samples from other samples in the panel and, in particular, normal adjacent lung tissue. Moreover, therapeutic modulation of this gene,

through the use of small molecule drugs, antibodies or protein therapeutics might be beneficial in the treatment of lung cancer.

Panel 3D Summary: Ag2256 The expression of the CG55690-01 gene appears to be highest in a sample derived from a lung cancer cell line (NCI-H146)(CT=30). In addition, there is a cluster of lung cancer cell lines that appear to be expressing this gene, consistent with expression seen in previous panels. Thus, the expression of this gene could be used to distinguish NCI-H146 cells from other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics might be beneficial in the treatment of lung cancer.

Panels 4.1D and 4D Summary: Ag4933 The CG55690-01 gene, a frizzled 9 homolog, is expressed at moderate levels in kidney (CT=31.31) and thymus (CT=32.69). Expression was analyzed independently with panel 4D and found to be at low levels in colon (CT=33.55) and lung (CT=34.7). Therefore, antibodies or small molecule antagonists that block the function of the CG55690-01 product may be useful to reduce or eliminate the symptoms in patients with diseases of kidney, thymus, colon, and lung.

Panel 5 Islet Summary: Ag2256 Expression of the CG55690-01 gene is low/undetectable in all samples on this panel (CTs>35). (Data not shown.)

NOV1 (CG56008-01)

Expression of gene CG56008-01 was assessed using the primer-probe set Ag2169, described in Table 35A. Results of the RTQ-PCR runs are shown in Tables 35B, 35C, 35D and 35E.

Table 35A. Probe Name Ag2169

Primers	Sequences	Length	Start Position
Forward	5'-cccgaaaaggctttatgtattc-3' (SEQ ID NO:201)	22	856
Probe	TET-5'-cagaaacacaaatgaaaatcctcagga-3'-TAMRA (SEQ ID NO:202)	27	878
Reverse	5'-tgtcagtagctttgatgcattg-3' (SEQ ID NO:203)	22	911

Table 35B. Panel 1.3D

Tissue Name	Rel. Exp.(%) Ag2169, Run 149923246	Rel. Exp.(%) Ag2169, Run 151268473	Tissue Name	Rel. Exp.(%) Ag2169, Run 149923246	Rel. Exp.(%) Ag2169, Run 151268473
Liver adenocarcinoma	1.8	2.0	Kidney (fetal)	1.1	0.8
Pancreas	1.0	0.4	Renal ca. 786-0	2.6	1.7
Pancreatic ca. CAPAN 2	1.0	1.0	Renal ca. A498	4.2	3.2

Adrenal gland	0.8	0.6	Renal ca. RXF 393	1.2	0.8
Thyroid	2.0	0.9	Renal ca. ACHN	2.6	2.7
Salivary gland	1.2	0.8	Renal ca. UO-31	3.3	2.4
Pituitary gland	3.1	2.2	Renal ca. TK-10	2.0	1.5
Brain (fetal)	2.2	1.7	Liver	0.1	0.1
Brain (whole)	2.6	2.1	Liver (fetal)	0.5	0.3
Brain (amygdala)	2.0	1.1	Liver ca. (hepatoblast) HepG2	1.5	1.3
Brain (cerebellum)	1.4	0.9	Lung	0.8	0.6
Brain (hippocampus)	6.1	4.5	Lung (fetal)	1.5	1.5
Brain (substantia nigra)	0.5	0.8	Lung ca. (small cell) LX-1	1.0	0.7
Brain (thalamus)	2.5	2.0	Lung ca. (small cell) NCI-H69	10.0	6.3
Cerebral Cortex	2.8	3.1	Lung ca. (s.cell var.) SHP-77	3.9	4.9
Spinal cord	1.6	1.4	Lung ca. (large cell)NCI-H460	1.3	1.2
glio/astro U87-MG	1.2	0.8	Lung ca. (non-sm. cell) A549	0.9	0.6
glio/astro U-118-MG	12.0	9.3	Lung ca. (non-s.cell) NCI-H23	5.4	0.0
astrocytoma SW1783	2.8	3.0	Lung ca. (non-s.cell) HOP-62	1.8	2.0
neuro*; met SK-N-AS	10.7	6.7	Lung ca. (non-s.cl) NCI-H522	1.8	1.2
astrocytoma SF-539	1.7	1.5	Lung ca. (squam.) SW 900	1.2	0.8
astrocytoma SNB-75	2.8	3.8	Lung ca. (squam.) NCI-H596	3.1	3.0
glioma SNB-19	1.0	0.9	Mammary gland	11.7	10.4
glioma U251	0.8	0.8	Breast ca.* (pl.ef) MCF-7	100.0	100.0

glioma SF-295	3.4	3.0	Breast ca.* (pl.ef) MDA-MB-231	2.5	2.1
Heart (fetal)	0.4	0.5	Breast ca.* (pl.ef) T47D	5.7	3.3
Heart	0.2	0.1	Breast ca. BT-549	4.5	3.6
Skeletal muscle (fetal)	1.2	1.4	Breast ca. MDA-N	2.6	2.8
Skeletal muscle	0.2	0.2	Ovary	2.0	1.3
Bone marrow	0.4	0.2	Ovarian ca. OVCAR-3	2.2	2.0
Thymus	0.3	0.3	Ovarian ca. OVCAR-4	0.3	0.2
Spleen	1.1	0.8	Ovarian ca. OVCAR-5	0.6	0.5
Lymph node	0.8	0.5	Ovarian ca. OVCAR-8	1.6	0.9
Colorectal	0.3	0.2	Ovarian ca. IGROV-1	0.8	0.5
Stomach	1.5	0.8	Ovarian ca.* (ascites) SK-OV-3	4.0	3.2
Small intestine	0.9	0.5	Uterus	1.1	0.8
Colon ca. SW480	1.6	1.2	Placenta	3.4	2.1
Colon ca.* SW620(SW480 met)	0.7	0.5	Prostate	5.5	4.6
Colon ca. HT29	0.8	0.6	Prostate ca.* (bone met)PC-3	2.0	1.3
Colon ca. HCT-116	4.2	3.1	Testis	1.9	1.6
Colon ca. CaCo-2	0.9	1.1	Melanoma Hs688(A).T	4.8	4.8
Colon ca. tissue(ODO3866)	1.3	1.2	Melanoma* (met) Hs688(B).T	6.2	5.2
Colon ca. HCC-2998	2.1	1.6	Melanoma UACC-62	0.3	0.3
Gastric ca.* (liver met) NCI-N87	2.0	1.6	Melanoma M14	2.8	2.6
Bladder	1.0	0.6	Melanoma LOX IMVI	0.6	0.4
Trachea	1.6	1.6	Melanoma* (met) SK-	7.1	5.1

			MEL-5		
Kidney	0.5	0.5	Adipose	1.2	0.8

Table 35C. Panel 2D

Tissue Name	Rel. Exp.(%) Ag2169, Run 148722818	Rel. Exp.(%) Ag2169, Run 149923296	Tissue Name	Rel. Exp.(%) Ag2169, Run 148722818	Rel. Exp.(%) Ag2169, Run 149923296
Normal Colon	3.2	3.1	Kidney Margin 8120608	0.3	0.2
CC Well to Mod Diff (ODO3866)	0.6	0.5	Kidney Cancer 8120613	0.4	0.4
CC Margin (ODO3866)	0.2	0.4	Kidney Margin 8120614	0.2	0.2
CC Gr.2 rectosigmoid (ODO3868)	0.1	0.2	Kidney Cancer 9010320	0.8	0.9
CC Margin (ODO3868)	0.2	0.1	Kidney Margin 9010321	0.5	0.6
CC Mod Diff (ODO3920)	0.2	0.2	Normal Uterus	0.0	0.4
CC Margin (ODO3920)	0.3	0.2	Uterus Cancer 064011	1.8	1.9
CC Gr.2 ascend colon (ODO3921)	1.0	1.1	Normal Thyroid	1.4	1.8
CC Margin (ODO3921)	0.3	0.4	Thyroid Cancer 064010	1.7	1.9
CC from Partial Hepatectomy (ODO4309) Mets	1.6	1.8	Thyroid Cancer A302152	0.9	0.9
Liver Margin (ODO4309)	0.5	0.4	Thyroid Margin A302153	1.5	1.5
Colon mets to lung (OD04451-01)	0.2	0.2	Normal Breast	3.9	4.9
Lung Margin (OD04451-02)	0.4	0.3	Breast Cancer (OD04566)	19.8	26.2
Normal Prostate 6546-1	7.7	7.9	Breast Cancer (OD04590-01)	46.7	45.7
Prostate Cancer (OD04410)	15.1	18.7	Breast Cancer Mets	43.2	57.8

			(OD04590-03)		
Prostate Margin (OD04410)	7.4	7.9	Breast Cancer Metastasis (OD04655-05)	**100.0**	**100.0**
Prostate Cancer (OD04720-01)	3.4	4.0	Breast Cancer 064006	2.4	2.7
Prostate Margin (OD04720-02)	6.7	7.3	Breast Cancer 1024	2.5	2.3
Normal Lung 061010	1.4	1.6	Breast Cancer 9100266	41.2	45.7
Lung Met to Muscle (ODO4286)	1.4	1.5	Breast Margin 9100265	5.0	5.7
Muscle Margin (ODO4286)	0.7	0.7	Breast Cancer A209073	4.0	5.4
Lung Malignant Cancer (OD03126)	1.7	2.0	Breast Margin A2090734	4.1	6.8
Lung Margin (OD03126)	1.1	1.3	Normal Liver	0.2	0.3
Lung Cancer (OD04404)	2.0	2.6	Liver Cancer 064003	0.2	0.2
Lung Margin (OD04404)	1.0	1.2	Liver Cancer 1025	0.2	0.1
Lung Cancer (OD04565)	1.0	1.1	Liver Cancer 1026	0.3	0.2
Lung Margin (OD04565)	0.5	0.5	Liver Cancer 6004-T	0.2	0.2
Lung Cancer (OD04237-01)	3.1	3.4	Liver Tissue 6004-N	0.5	0.5
Lung Margin (OD04237-02)	0.9	1.0	Liver Cancer 6005-T	0.2	0.2
Ocular Mel Met to Liver (ODO4310)	3.7	4.1	Liver Tissue 6005-N	0.1	0.1
Liver Margin (ODO4310)	0.2	0.4	Normal Bladder	1.5	1.4
Melanoma Mets to Lung (OD04321)	3.5	4.0	Bladder Cancer 1023	0.3	0.3
Lung Margin (OD04321)	0.9	1.4	Bladder Cancer A302173	1.7	1.8
Normal Kidney	2.5	3.1	Bladder Cancer (OD04718-01)	3.0	3.3
Kidney Ca,	2.8	2.8	Bladder	2.9	1.4

Nuclear grade 2 (OD04338)			Normal Adjacent (OD04718-03)		
Kidney Margin (OD04338)	1.8	1.9	Normal Ovary	0.3	0.3
Kidney Ca Nuclear grade 1/2 (OD04339)	0.7	0.6	Ovarian Cancer 064008	3.3	3.0
Kidney Margin (OD04339)	1.4	1.4	Ovarian Cancer (OD04768-07)	3.1	3.1
Kidney Ca, Clear cell type (OD04340)	2.5	3.1	Ovary Margin (OD04768-08)	0.4	0.5
Kidney Margin (OD04340)	1.8	1.9	Normal Stomach	0.5	0.5
Kidney Ca, Nuclear grade 3 (OD04348)	1.0	0.9	Gastric Cancer 9060358	0.2	0.2
Kidney Margin (OD04348)	1.5	1.7	Stomach Margin 9060359	0.4	0.4
Kidney Cancer (OD04622-01)	0.9	0.9	Gastric Cancer 9060395	0.8	0.7
Kidney Margin (OD04622-03)	0.2	0.1	Stomach Margin 9060394	0.5	0.5
Kidney Cancer (OD04450-01)	1.1	1.0	Gastric Cancer 9060397	1.0	0.9
Kidney Margin (OD04450-03)	1.4	1.5	Stomach Margin 9060396	0.1	0.1
Kidney Cancer 8120607	0.5	0.4	Gastric Cancer 064005	1.0	0.8

Table 35D. Panel 3D

Tissue Name	Rel. Exp.(%) Ag2169, Run 170745433	Tissue Name	Rel. Exp.(%) Ag2169, Run 170745433
Daoy- Medulloblastoma	3.2	Ca Ski- Cervical epidermoid carcinoma (metastasis)	11.6
TE671- Medulloblastoma	1.2	ES-2- Ovarian clear cell carcinoma	4.4
D283 Med- Medulloblastoma	19.2	Ramos- Stimulated with PMA/ionomycin 6h	5.0
PFSK-1- Primitive Neuroectodermal	16.4	Ramos- Stimulated with PMA/ionomycin 14h	6.2

XF-498- CNS	15.5	MEG-01- Chronic myelogenous leukemia (megokaryoblast)	3.3
SNB-78- Glioma	20.3	Raji- Burkitt's lymphoma	1.2
SF-268- Glioblastoma	2.5	Daudi- Burkitt's lymphoma	4.6
T98G- Glioblastoma	5.4	U266- B-cell plasmacytoma	11.4
SK-N-SH- Neuroblastoma (metastasis)	16.5	CA46- Burkitt's lymphoma	2.1
SF-295- Glioblastoma	7.2	RL- non-Hodgkin's B-cell lymphoma	0.6
Cerebellum	6.1	JM1- pre-B-cell lymphoma	3.0
Cerebellum	2.5	Jurkat- T cell leukemia	11.7
NCI-H292- Mucoepidermoid lung carcinoma	32.8	TF-1- Erythroleukemia	2.9
DMS-114- Small cell lung cancer	9.1	HUT 78- T-cell lymphoma	2.9
DMS-79- Small cell lung cancer	**100.0**	U937- Histiocytic lymphoma	4.2
NCI-H146- Small cell lung cancer	31.6	KU-812- Myelogenous leukemia	1.3
NCI-H526- Small cell lung cancer	25.0	769-P- Clear cell renal carcinoma	11.3
NCI-N417- Small cell lung cancer	5.0	Caki-2- Clear cell renal carcinoma	8.0
NCI-H82- Small cell lung cancer	10.1	SW 839- Clear cell renal carcinoma	2.6
NCI-H157- Squamous cell lung cancer (metastasis)	12.9	G401- Wilms' tumor	4.1
NCI-H1155- Large cell lung cancer	17.7	Hs766T- Pancreatic carcinoma (LN metastasis)	12.3
NCI-H1299- Large cell lung cancer	15.0	CAPAN-1- Pancreatic adenocarcinoma (liver metastasis)	2.2
NCI-H727- Lung carcinoid	4.0	SU86.86- Pancreatic carcinoma (liver metastasis)	3.2
NCI-UMC-11- Lung carcinoid	21.3	BxPC-3- Pancreatic adenocarcinoma	4.8
LX-1- Small cell lung cancer	6.1	HPAC- Pancreatic adenocarcinoma	10.4
Colo-205- Colon cancer	3.9	MIA PaCa-2- Pancreatic carcinoma	3.4
KM12- Colon cancer	6.1	CFPAC-1- Pancreatic ductal adenocarcinoma	22.1

KM20L2- Colon cancer	3.5	PANC-1- Pancreatic epithelioid ductal carcinoma	14.1
NCI-H716- Colon cancer	8.8	T24- Bladder carcinma (transitional cell)	10.7
SW-48- Colon adenocarcinoma	4.2	5637- Bladder carcinoma	11.8
SW1116- Colon adenocarcinoma	6.3	HT-1197- Bladder carcinoma	4.2
LS 174T- Colon adenocarcinoma	3.4	UM-UC-3- Bladder carcinma (transitional cell)	2.5
SW-948- Colon adenocarcinoma	0.8	A204- Rhabdomyosarcoma	4.3
SW-480- Colon adenocarcinoma	3.2	HT-1080- Fibrosarcoma	15.3
NCI-SNU-5- Gastric carcinoma	1.4	MG-63- Osteosarcoma	3.5
KATO III- Gastric carcinoma	11.0	SK-LMS-1- Leiomyosarcoma (vulva)	10.2
NCI-SNU-16- Gastric carcinoma	7.2	SJRH30- Rhabdomyosarcoma (met to bone marrow)	3.1
NCI-SNU-1- Gastric carcinoma	9.2	A431- Epidermoid carcinoma	4.8
RF-1- Gastric adenocarcinoma	6.1	WM266-4- Melanoma	11.0
RF-48- Gastric adenocarcinoma	9.5	DU 145- Prostate carcinoma (brain metastasis)	0.0
MKN-45- Gastric carcinoma	12.8	MDA-MB-468- Breast adenocarcinoma	5.5
NCI-N87- Gastric carcinoma	4.4	SCC-4- Squamous cell carcinoma of tongue	0.0
OVCAR-5- Ovarian carcinoma	0.5	SCC-9- Squamous cell carcinoma of tongue	0.0
RL95-2- Uterine carcinoma	5.4	SCC-15- Squamous cell carcinoma of tongue	0.0
HelaS3- Cervical adenocarcinoma	11.7	CAL 27- Squamous cell carcinoma of tongue	7.3

Table 35E. Panel 4D

Tissue Name	Rel. Exp.(%) Ag2169, Run 148725333	Rel. Exp.(%) Ag2169, Run 163923835	Tissue Name	Rel. Exp.(%) Ag2169, Run 148725333	Rel. Exp.(%) Ag2169, Run 163923835
Secondary Th1 act	12.9	10.3	HUVEC IL-1beta	4.1	4.1
Secondary Th2 act	15.3	13.9	HUVEC IFN gamma	3.5	2.4
Secondary Tr1 act	17.6	16.3	HUVEC TNF	9.3	5.1

			alpha + IFN gamma		
Secondary Th1 rest	2.2	1.5	HUVEC TNF alpha + IL4	4.8	5.4
Secondary Th2 rest	2.9	2.7	HUVEC IL-11	1.2	0.9
Secondary Tr1 rest	3.7	3.7	Lung Microvascular EC none	4.2	3.2
Primary Th1 act	18.7	22.7	Lung Microvascular EC TNFalpha + IL-1beta	7.3	5.4
Primary Th2 act	23.8	10.7	Microvascular Dermal EC none	4.3	4.8
Primary Tr1 act	24.3	17.6	Microsvasular Dermal EC TNFalpha + IL-1beta	7.0	6.5
Primary Th1 rest	17.4	19.6	Bronchial epithelium TNFalpha + IL1beta	24.1	40.6
Primary Th2 rest	6.0	7.6	Small airway epithelium none	15.7	11.8
Primary Tr1 rest	6.2	6.0	Small airway epithelium TNFalpha + IL-1beta	**100.0**	**100.0**
CD45RA CD4 lymphocyte act	12.9	8.4	Coronery artery SMC rest	18.9	12.6
CD45RO CD4 lymphocyte act	21.2	12.8	Coronery artery SMC TNFalpha + IL-1beta	13.9	9.3
CD8 lymphocyte act	8.9	7.8	Astrocytes rest	16.7	13.6
Secondary CD8 lymphocyte rest	9.5	9.1	Astrocytes TNFalpha + IL-1beta	15.2	9.2
Secondary CD8 lymphocyte act	5.4	6.8	KU-812 (Basophil) rest	1.1	0.9
CD4 lymphocyte none	1.6	1.4	KU-812 (Basophil) PMA/ionomycin	5.5	3.9
2ry Th1/Th2/Tr1_anti-CD95 CH11	3.8	6.2	CCD1106 (Keratinocytes) none	14.8	10.3
LAK cells rest	8.4	6.8	CCD1106	2.9	5.0

			(Keratinocytes) TNFalpha + IL-1beta		
LAK cells IL-2	8.2	10.3	Liver cirrhosis	0.9	0.9
LAK cells IL-2+IL-12	13.3	9.5	Lupus kidney	1.5	1.3
LAK cells IL-2+IFN gamma	17.1	13.4	NCI-H292 none	30.8	21.6
LAK cells IL-2+ IL-18	14.7	12.1	NCI-H292 IL-4	40.6	31.4
LAK cells PMA/ionomycin	9.2	7.4	NCI-H292 IL-9	35.8	28.9
NK Cells IL-2 rest	7.0	5.0	NCI-H292 IL-13	17.7	13.3
Two Way MLR 3 day	7.3	7.0	NCI-H292 IFN gamma	23.8	17.3
Two Way MLR 5 day	7.3	6.8	HPAEC none	2.0	1.4
Two Way MLR 7 day	6.2	5.9	HPAEC TNF alpha + IL-1 beta	9.6	5.3
PBMC rest	1.9	2.1	Lung fibroblast none	15.2	11.4
PBMC PWM	41.2	47.3	Lung fibroblast TNF alpha + IL-1 beta	15.3	10.4
PBMC PHA-L	14.8	11.1	Lung fibroblast IL-4	37.4	31.9
Ramos (B cell) none	9.7	8.8	Lung fibroblast IL-9	23.2	17.8
Ramos (B cell) ionomycin	47.6	40.6	Lung fibroblast IL-13	23.5	19.5
B lymphocytes PWM	71.2	70.2	Lung fibroblast IFN gamma	38.7	32.3
B lymphocytes CD40L and IL-4	9.1	9.9	Dermal fibroblast CCD1070 rest	36.3	33.7
EOL-1 dbcAMP	9.8	6.8	Dermal fibroblast CCD1070 TNF alpha	46.3	48.3
EOL-1 dbcAMP PMA/ionomycin	7.2	5.3	Dermal fibroblast CCD1070 IL-1 beta	18.6	18.8
Dendritic cells none	9.6	7.7	Dermal fibroblast IFN gamma	14.5	14.4
Dendritic cells LPS	18.3	10.5	Dermal fibroblast IL-4	29.9	29.3
Dendritic cells anti-CD40	12.2	9.2	IBD Colitis 2	0.2	0.4

Monocytes rest	5.7	4.0	IBD Crohn's	0.5	0.4
Monocytes LPS	8.0	4.6	Colon	4.9	4.1
Macrophages rest	12.3	11.8	Lung	8.1	8.6
Macrophages LPS	4.8	4.1	Thymus	14.8	16.4
HUVEC none	3.9	2.7	Kidney	7.1	5.3
HUVEC starved	8.8	6.3			

Panel 1.3D Summary: Ag2169 The expression of the CG56008-01 gene was assessed in two independent runs in panel 1.3D with excellent concordance. The expression of this gene is highest in a sample derived from a breast cancer cell line (MCF-7)(CTs=25-26). Thus, the expression of this gene could be used to distinguish MCF-7 cells from the other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics may be beneficial in the treatment of breast cancer.

This tissue is moderately expressed in a variety of metabolic tissues, including pancreas, adrenal, thyroid, pituitary, adult and fetal heart, fetal liver and adipose.

Thus, this gene product may be a monoclonal antibody target for the treatment of metabolic and endocrine disease, including obesity and Types 1 and 2 diabetes. As a putative zinc transporter, this gene may also be a potential target for the enhancement of insulin secretion and sensitivity in all forms of Type 2 diabetes. In addition, this gene is differentially expressed in fetal (CTs=31-32) vs adult skeletal muscle (CTs=34-35), and may be useful for the identification of the fetal source of this tissue. Furthermore, the relative overexpression of this gene in fetal skeletal muscle suggests that the protein product may enhance muscular growth or development in the fetus and thus may also act in a regenerative capacity in the adult. Therefore, therapeutic modulation of the protein encoded by this gene could be useful in treatment of muscle related diseases. More specifically, treatment of weak or dystrophic muscle with the protein encoded by this gene could restore muscle mass or function.

Among tissues of CNS origin, this gene is expressed at moderate levels in all regions examined. This gene, a LIV-1 homolog, may be involved in zinc homeostasis. Zinc is critical to brain functions as it may serve as an endogenous neuromodulator in synaptic neurotransmission. Thus, this gene would be a drug target for the treatment of learning deficiencies and seizure disorders associated with improper zinc trafficking.

References:

Tang X, Shay NF. Zinc has an insulin-like effect on glucose transport mediated by phosphoinositol-3-kinase and Akt in 3T3-L1 fibroblasts and adipocytes. J Nutr. 2001 May;131(5):1414-20.

Zinc has insulin-like effects on cells, including promotion of both lipogenesis and glucose
5   transport. The relationship between zinc and the stimulation of glucose transport is unclear. We hypothesize that zinc affects the insulin-signaling pathway. In this study, the effect of zinc on glucose transport and insulin signaling was examined in 3T3-L1-preadipocytes and - adipocytes. Treatment of cells with up to 200 micromol/L zinc significantly increased glucose transport (P < 0.05). The effect of zinc on adipocytes was greater than on preadipocytes, and
10   the effect of zinc plus insulin was greater than that of either insulin or zinc alone. Cytochalasin D, which disrupts actin filaments, attenuated the increase of glucose transport induced by zinc or insulin (P < 0.05). At 100 nmol/L, wortmannin, the phosphoinositide (PI) 3-kinase inhibitor, decreased basal glucose transport and blocked zinc-stimulated glucose transport in both cell types (P < 0.05). H7, an inhibitor of protein kinase C, did not reduce basal glucose
15   transport but decreased zinc-induced glucose transport (P < 0.05). Zinc increased tyrosine phosphorylation of the insulin receptor beta subunit of both preadipocytes and adipocytes after 5-10 min of treatment (P < 0.05). Zinc at 200 micromol/L did not affect tyrosine phosphorylation of insulin receptor substrate (IRS)-1 or -2; further, there was no effect of zinc on the association of the p85 subunit of PI 3-kinase and IRS-1. Zinc significantly increased
20   serine-473 phosphorylation of Akt in both preadipocytes and adipocytes (P] 0.05). The PI 3- kinase inhibitor, wortmannin, totally blocked the effect of zinc on Akt activation. Hence, it appears that zinc can induce an increase in glucose transport into cells and potentiate insulin- induced glucose transport, likely acting through the insulin-signaling pathway.

PMID: 11340092

25   Taylor KM. LIV-1 breast cancer protein belongs to new family of histidine-rich membrane proteins with potential to control intracellular Zn2+ homeostasis. IUBMB Life 2000 Apr;49(4):249-53

Investigation of the protein product of the oestrogen-regulated gene LIV-1, implicated in metastatic breast cancer, has revealed 10 protein sequences of unknown function that belong
30   to a new family with potential to control intracellular Zn2+ homeostasis. Sequence alignment highlights the similarity in transmembrane domains and extramembrane charged residues, indicating potential ion-transport ability. This family has a novel highly conserved motif of 66 residues, including a transmembrane domain and a catalytic zinc-binding sequence of zinc

metalloproteases, containing conserved (indicated in bold type) proline and glutamine residues, HEXPHEXGD. These proteins contain more plentiful histidine-rich repeats than zinc transporters, suggesting an ability to bind or transport zinc across membranes. I propose that these 11 proteins form a new family with the potential to control intracellular Zn2+

5   homeostasis.

Takeda A. Movement of zinc and its functional significance in the brain. Brain Res Brain Res Rev 2000 Dec;34(3):137-48

Zinc, an essential nutrient, is supplied to the brain via both the blood-brain and blood-cerebrospinal fluid barriers. Zinc is most concentrated in the limbic system, i.e. the

10   hippocampus and amygdala, zinc-containing glutaminergic neuron-rich areas. A large portion of zinc serves the function of zinc metalloproteins in neurons and glial cells. In zinc-containing glutaminergic neurons, vesicular zinc, probably ionic zinc, may serve as an endogenous neuromodulator in synaptic neurotransmission. Vesicular zinc is dynamically coupled to the electrophysiological activity of zinc-containing glutaminergic neurons. Dietary

15   zinc deprivation may influence zinc homeostasis in the brain, resulting in brain dysfunction such as learning impairment. Excessive excitation of zinc-containing glutaminergic neurons causes a decrease in vesicular zinc, and the decrease might be associated with the susceptibility to seizure. Alteration of zinc levels released into the synaptic cleft may influence neurotransmission in zinc-containing glutaminergic synapses. Therefore, zinc homeostasis in

20   the presynaptic vesicle is important for the function of zinc-containing glutaminergic neurons

Panel 2D Summary: Ag2169 The expression of the CG56008-01 gene was assessed in two independent runs in panel 2D with excellent concordance. It appears that the expression of this gene is highest in a sample derived from a breast cancer (CTs=23-24), consistent with expression in Panel 1.3D. In addition, there is a strong cluster of breast cancers expressing this

25   gene, while expression of this gene in other tissues is almost absent, with the exception of a cluster of prostate derived samples. Thus, the expression of this gene could be used to distinguish breast cancer samples from the other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics may be beneficial in the treatment of breast cancer.

30   **Panel 3D Summary:** Ag2169

The expression of the CG56008-01 gene appears to be highest is a sample derived from a lung cancer cell line (DMS 79)(CT=27.8). In addition, there appears to be significant levels of expression in a cluster of other lung cancer cell lines. Thus, the expression of this gene could

be used to distinguish DMS 79 cells from other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, antibodies or protein therapeutics may be beneficial in the treatment of lung cancer.

Panel 4D Summary: Ag2169 Two experiments with the same probe and primer set show highest expression of the CG56008-01 gene, a LIV-I homolog in small airway epithelium stimulated with TNF-alpha and IL-1beta (CTs=27). Moderate levels of expression are also seen in pokeweed mitogen-activated peripheral blood mononuclear cells (mainly B cells), ionomycin-activated Ramos B cell, pokeweed mitogen-activated purified peripheral blood B lymphocytes, B lymphocytes activated with CD40L and IL-4, and a number of cytokine-activated and resting cells including NCI-H292 pulmonary mucoepidermoid epithelial cells, lung fibroblasts, and dermal fibroblasts. Based on these levels of expression in cytokine-activated B cells and cells in lung and skin, small molecule antagonists that block the function of this gene product may be useful as therapeutics that reduce or eliminate the symptoms in patients with autoimmune and inflammatory diseases in which activated B cells present antigens in the generation of the aberrant immune response, including Crohn's disease, ulcerative colitis, multiple sclerosis, chronic obstructive pulmonary disease, asthma, emphysema, rheumatoid arthritis, or psoriasis.

NOV1 (CG56008-03)

Expression of gene CG56008-03 was assessed using the primer-probe set Ag4704, described in Table 36A.

Table 36A. Probe Name Ag4704

Primers	Sequences	Length	Start Position
Forward	5'-gctttgggtttggaattatg-3' (SEQ ID NO:204)	22	962
Probe	TET-5'-tccatatttgaacataaaatcgtgtttcg-3'-TAMRA (SEQ ID NO:205)	29	993
Reverse	5'-gtggtgatgatggagaattgaa-3' (SEQ ID NO:206)	22	1029

CNS_neurodegeneration_v1.0 Summary: Ag4704 Expression of the CG56008-03 gene is low/undetectable in all samples on this panel (CTs>35). (Data not shown.) The amp plot indicates that there is a high probability of a probe failure.

General_screening_panel_v1.4 Summary: Ag4704 Expression of the CG56008-03 gene is low/undetectable in all samples on this panel (CTs>35). (Data not shown.) The amp plot indicates that there is a high probability of a probe failure.

Panel 4.1D Summary: Ag4704 Expression of the CG56008-03 gene is low/undetectable in all samples on this panel (CTs>35). (Data not shown.) The amp plot indicates that there is a high probability of a probe failure.

NOV8 (CG56006-01)

377

Expression of gene CG56006-01 was assessed using the primer-probe set Ag1437, described in Table 37A. Results of the RTQ-PCR runs are shown in Tables 37B, 37C, 37D and 37E.

Table 37A. Probe Name Ag1437

Primers	Sequences	Length	Start Position
Forward	5'-aggacagaacacctaggtgctt-3' (SEQ ID NO:207)	22	329
Probe	TET-5'-ctcttcaggtccccaggaacccct-3'-TAMRA (SEQ ID NO:208)	24	284
Reverse	5'-cctaatgcccacctcctaatag-3' (SEQ ID NO:209)	22	262

Table 37B. Panel 1.2

Tissue Name	Rel. Exp.(%) Ag1437, Run 138297722	Tissue Name	Rel. Exp.(%) Ag1437, Run 138297722
Endothelial cells	0.0	Renal ca. 786-0	0.0
Heart (Fetal)	4.5	Renal ca. A498	0.1
Pancreas	1.8	Renal ca. RXF 393	0.1
Pancreatic ca. CAPAN 2	0.0	Renal ca. ACHN	0.0
Adrenal Gland	0.4	Renal ca. UO-31	0.0
Thyroid	0.1	Renal ca. TK-10	0.1
Salivary gland	1.2	Liver	46.7
Pituitary gland	0.0	Liver (fetal)	12.5
Brain (fetal)	0.0	Liver ca. (hepatoblast) HepG2	18.8
Brain (whole)	0.1	Lung	0.2
Brain (amygdala)	0.1	Lung (fetal)	0.2
Brain (cerebellum)	0.1	Lung ca. (small cell) LX-1	0.2
Brain (hippocampus)	0.4	Lung ca. (small cell) NCI-H69	0.1
Brain (thalamus)	0.4	Lung ca. (s.cell var.) SHP-77	0.1
Cerebral Cortex	0.5	Lung ca. (large cell)NCI-H460	0.0
Spinal cord	0.0	Lung ca. (non-sm. cell) A549	0.2
glio/astro U87-MG	0.0	Lung ca. (non-s.cell) NCI-H23	0.0
glio/astro U-118-MG	0.0	Lung ca. (non-s.cell) HOP-62	0.1
astrocytoma SW1783	0.0	Lung ca. (non-s.cl) NCI-H522	0.5
neuro*; met SK-N-AS	0.0	Lung ca. (squam.) SW 900	3.1
astrocytoma SF-539	0.0	Lung ca. (squam.) NCI-H596	0.1
astrocytoma SNB-75	0.0	Mammary gland	1.0

glioma SNB-19	0.2	Breast ca.* (pl.ef) MCF-7	0.2
glioma U251	0.0	Breast ca.* (pl.ef) MDA-MB-231	0.0
glioma SF-295	0.1	Breast ca.* (pl. ef) T47D	5.4
Heart	1.3	Breast ca. BT-549	0.0
Skeletal Muscle	0.8	Breast ca. MDA-N	0.0
Bone marrow	0.0	Ovary	0.0
Thymus	0.0	Ovarian ca. OVCAR-3	0.9
Spleen	0.2	Ovarian ca. OVCAR-4	0.2
Lymph node	0.0	Ovarian ca. OVCAR-5	1.1
Colorectal Tissue	0.1	Ovarian ca. OVCAR-8	0.0
Stomach	1.3	Ovarian ca. IGROV-1	0.0
Small intestine	0.2	Ovarian ca. (ascites) SK-OV-3	0.1
Colon ca. SW480	0.0	Uterus	0.1
Colon ca.* SW620 (SW480 met)	0.2	Placenta	0.0
Colon ca. HT29	0.1	Prostate	3.7
Colon ca. HCT-116	0.1	Prostate ca.* (bone met) PC-3	0.0
Colon ca. CaCo-2	4.9	Testis	0.0
Colon ca. Tissue (ODO3866)	0.0	Melanoma Hs688(A).T	0.0
Colon ca. HCC-2998	2.7	Melanoma* (met) Hs688(B).T	0.0
Gastric ca.* (liver met) NCI-N87	0.2	Melanoma UACC-62	0.7
Bladder	15.4	Melanoma M14	0.6
Trachea	0.0	Melanoma LOX IMVI	0.0
Kidney	**100.0**	Melanoma* (met) SK-MEL-5	0.0
Kidney (fetal)	1.9		

Table 37C. Panel 1.3D

Tissue Name	Rel. Exp.(%) Ag1437, Run 146124971	Rel. Exp.(%) Ag1437, Run 151531145	Tissue Name	Rel. Exp.(%) Ag1437, Run 146124971	Rel. Exp.(%) Ag1437, Run 151531145
Liver	0.0	0.0	Kidney (fetal)	26.8	28.5

adenocarcinoma					
Pancreas	34.4	67.4	Renal ca. 786-0	0.0	0.0
Pancreatic ca. CAPAN 2	0.0	0.0	Renal ca. A498	0.9	1.4
Adrenal gland	0.4	2.0	Renal ca. RXF 393	0.0	0.0
Thyroid	9.3	9.1	Renal ca. ACHN	0.4	0.0
Salivary gland	1.2	1.8	Renal ca. UO-31	0.0	0.0
Pituitary gland	5.3	6.0	Renal ca. TK-10	0.3	0.6
Brain (fetal)	0.0	0.0	Liver	66.9	88.9
Brain (whole)	0.8	7.3	Liver (fetal)	66.0	**100.0**
Brain (amygdala)	2.7	0.0	Liver ca. (hepatoblast) HepG2	**100.0**	90.8
Brain (cerebellum)	3.1	1.7	Lung	15.6	21.0
Brain (hippocampus)	4.6	14.5	Lung (fetal)	14.8	8.1
Brain (substantia nigra)	0.3	0.7	Lung ca. (small cell) LX-1	1.2	1.8
Brain (thalamus)	1.4	3.7	Lung ca. (small cell) NCI-H69	0.4	0.0
Cerebral Cortex	1.5	2.5	Lung ca. (s.cell var.) SHP-77	0.7	3.2
Spinal cord	3.0	1.2	Lung ca. (large cell)NCI-H460	0.0	0.0
glio/astro U87-MG	0.0	0.0	Lung ca. (non-sm. cell) A549	0.4	0.0
glio/astro U-118-MG	0.4	0.0	Lung ca. (non-s.cell) NCI-H23	0.0	0.0
astrocytoma SW1783	0.0	0.0	Lung ca. (non-s.cell) HOP-62	0.8	0.0
neuro*; met SK-N-AS	0.0	0.0	Lung ca. (non-s.cl) NCI-H522	0.0	1.1
astrocytoma SF-539	0.8	1.2	Lung ca. (squam.) SW 900	9.4	8.0
astrocytoma SNB-	9.4	5.4	Lung ca.	0.0	0.0

75			(squam.) NCI-H596		
glioma SNB-19	0.2	0.0	Mammary gland	13.3	8.7
glioma U251	0.0	0.0	Breast ca.* (pl.ef) MCF-7	0.7	0.0
glioma SF-295	0.3	0.6	Breast ca.* (pl.ef) MDA-MB-231	0.7	0.0
Heart (fetal)	3.8	0.3	Breast ca.* (pl.ef) T47D	22.4	18.4
Heart	0.8	0.5	Breast ca. BT-549	0.0	0.0
Skeletal muscle (fetal)	12.5	21.2	Breast ca. MDA-N	0.0	0.0
Skeletal muscle	1.6	0.0	Ovary	0.4	1.2
Bone marrow	0.7	0.0	Ovarian ca. OVCAR-3	3.0	4.2
Thymus	0.0	0.7	Ovarian ca. OVCAR-4	0.7	0.3
Spleen	3.1	5.1	Ovarian ca. OVCAR-5	4.6	0.5
Lymph node	1.1	0.5	Ovarian ca. OVCAR-8	0.4	0.0
Colorectal	2.6	2.4	Ovarian ca. IGROV-1	0.0	0.0
Stomach	47.0	52.9	Ovarian ca.* (ascites) SK-OV-3	0.4	0.0
Small intestine	2.0	0.0	Uterus	1.3	1.3
Colon ca. SW480	0.3	0.6	Placenta	0.7	2.7
Colon ca.* SW620(SW480 met)	2.6	1.1	Prostate	6.1	11.7
Colon ca. HT29	1.1	1.2	Prostate ca.* (bone met)PC-3	0.0	0.0
Colon ca. HCT-116	0.0	0.6	Testis	0.7	0.6
Colon ca. CaCo-2	42.6	36.9	Melanoma Hs688(A).T	0.0	0.0
Colon ca. tissue(ODO3866)	0.0	0.3	Melanoma* (met) Hs688(B).T	0.0	0.0
Colon ca. HCC-2998	7.8	7.8	Melanoma UACC-62	0.3	0.0

Gastric ca.* (liver met) NCI-N87	0.7	0.4	Melanoma M14	1.4	1.0
Bladder	20.0	30.6	Melanoma LOX IMVI	0.0	0.0
Trachea	1.6	1.1	Melanoma* (met) SK-MEL-5	0.0	0.0
Kidney	60.3	60.7	Adipose	2.7	0.0

Table 37D. Panel 2D

Tissue Name	Rel. Exp.(%) Ag1437, Run 144872207	Rel. Exp.(%) Ag1437, Run 145090527	Tissue Name	Rel. Exp.(%) Ag1437, Run 144872207	Rel. Exp.(%) Ag1437, Run 145090527
Normal Colon	1.3	1.0	Kidney Margin 8120608	20.3	17.6
CC Well to Mod Diff (ODO3866)	0.2	0.2	Kidney Cancer 8120613	16.5	7.2
CC Margin (ODO3866)	0.0	0.0	Kidney Margin 8120614	20.3	16.3
CC Gr.2 rectosigmoid (ODO3868)	0.3	0.2	Kidney Cancer 9010320	4.1	4.0
CC Margin (ODO3868)	0.2	0.0	Kidney Margin 9010321	32.1	30.1
CC Mod Diff (ODO3920)	0.0	0.0	Normal Uterus	0.0	0.0
CC Margin (ODO3920)	0.0	0.0	Uterus Cancer 064011	1.9	1.9
CC Gr.2 ascend colon (ODO3921)	0.0	0.0	Normal Thyroid	2.7	2.0
CC Margin (ODO3921)	1.0	0.0	Thyroid Cancer 064010	2.4	3.2
CC from Partial Hepatectomy (ODO4309) Mets	1.5	1.3	Thyroid Cancer A302152	5.3	8.4
Liver Margin (ODO4309)	30.4	22.7	Thyroid Margin A302153	2.8	2.0
Colon mets to lung (OD04451-01)	0.5	0.5	Normal Breast	2.2	1.6

Lung Margin (OD04451-02)	1.0	0.7	Breast Cancer (OD04566)	11.1	11.0
Normal Prostate 6546-1	5.1	5.4	Breast Cancer (OD04590-01)	15.8	14.3
Prostate Cancer (OD04410)	20.4	18.0	Breast Cancer Mets (OD04590-03)	25.7	52.5
Prostate Margin (OD04410)	1.5	2.0	Breast Cancer Metastasis (OD04655-05)	3.8	4.2
Prostate Cancer (OD04720-01)	8.5	7.4	Breast Cancer 064006	3.2	4.1
Prostate Margin (OD04720-02)	2.4	2.5	Breast Cancer 1024	7.1	5.3
Normal Lung 061010	3.6	3.7	Breast Cancer 9100266	10.7	11.0
Lung Met to Muscle (ODO4286)	0.0	0.3	Breast Margin 9100265	2.8	4.2
Muscle Margin (ODO4286)	4.9	2.4	Breast Cancer A209073	0.7	0.0
Lung Malignant Cancer (OD03126)	16.5	10.0	Breast Margin A2090734	3.2	1.8
Lung Margin (OD03126)	3.6	3.3	Normal Liver	37.4	29.5
Lung Cancer (OD04404)	1.4	0.9	Liver Cancer 064003	**100.0**	94.6
Lung Margin (OD04404)	3.2	2.9	Liver Cancer 1025	41.5	32.8
Lung Cancer (OD04565)	0.8	1.0	Liver Cancer 1026	9.9	9.7
Lung Margin (OD04565)	2.5	1.9	Liver Cancer 6004-T	87.1	**100.0**
Lung Cancer (OD04237-01)	2.1	2.7	Liver Tissue 6004-N	10.6	11.8
Lung Margin (OD04237-02)	1.8	1.6	Liver Cancer 6005-T	13.1	8.8
Ocular Mel Met to Liver (ODO4310)	2.1	1.8	Liver Tissue 6005-N	4.7	3.4
Liver Margin (ODO4310)	52.5	57.8	Normal Bladder	14.8	8.8
Melanoma Mets to Lung (OD04321)	1.7	0.8	Bladder Cancer 1023	2.1	1.4
Lung Margin	4.7	4.1	Bladder	0.6	0.5

(OD04321)			Cancer A302173		
Normal Kidney	74.7	65.1	Bladder Cancer (OD04718-01)	0.0	0.0
Kidney Ca, Nuclear grade 2 (OD04338)	21.6	19.6	Bladder Normal Adjacent (OD04718-03)	0.3	0.7
Kidney Margin (OD04338)	18.6	17.1	Normal Ovary	0.1	0.2
Kidney Ca Nuclear grade 1/2 (OD04339)	25.0	28.1	Ovarian Cancer 064008	2.9	3.8
Kidney Margin (OD04339)	73.7	67.4	Ovarian Cancer (OD04768-07)	0.3	0.5
Kidney Ca, Clear cell type (OD04340)	16.0	15.9	Ovary Margin (OD04768-08)	0.0	0.0
Kidney Margin (OD04340)	52.5	47.3	Normal Stomach	2.8	3.0
Kidney Ca, Nuclear grade 3 (OD04348)	0.1	0.0	Gastric Cancer 9060358	0.0	0.0
Kidney Margin (OD04348)	30.6	35.1	Stomach Margin 9060359	1.6	2.2
Kidney Cancer (OD04622-01)	2.2	2.8	Gastric Cancer 9060395	0.0	0.0
Kidney Margin (OD04622-03)	8.0	5.1	Stomach Margin 9060394	0.2	0.2
Kidney Cancer (OD04450-01)	10.4	10.9	Gastric Cancer 9060397	0.2	0.2
Kidney Margin (OD04450-03)	18.2	19.5	Stomach Margin 9060396	0.0	0.2
Kidney Cancer 8120607	1.5	0.3	Gastric Cancer 064005	0.0	0.5

Table 37E. Panel 4D

Tissue Name	Rel. Exp.(%) Ag1437, Run 146424179	Tissue Name	Rel. Exp.(%) Ag1437, Run 146424179
Secondary Th1 act	0.0	HUVEC IL-1beta	0.0
Secondary Th2 act	0.0	HUVEC IFN gamma	1.2

Secondary Tr1 act	0.0	HUVEC TNF alpha + IFN gamma	0.2
Secondary Th1 rest	0.0	HUVEC TNF alpha + IL4	0.0
Secondary Th2 rest	0.3	HUVEC IL-11	0.0
Secondary Tr1 rest	0.0	Lung Microvascular EC none	0.0
Primary Th1 act	0.0	Lung Microvascular EC TNFalpha + IL-1beta	0.0
Primary Th2 act	0.0	Microvascular Dermal EC none	0.0
Primary Tr1 act	0.0	Microsvasular Dermal EC TNFalpha + IL-1beta	0.2
Primary Th1 rest	0.0	Bronchial epithelium TNFalpha + IL1beta	0.0
Primary Th2 rest	0.0	Small airway epithelium none	0.0
Primary Tr1 rest	0.0	Small airway epithelium TNFalpha + IL-1beta	0.0
CD45RA CD4 lymphocyte act	0.0	Coronery artery SMC rest	3.9
CD45RO CD4 lymphocyte act	0.2	Coronery artery SMC TNFalpha + IL-1beta	0.0
CD8 lymphocyte act	0.0	Astrocytes rest	0.0
Secondary CD8 lymphocyte rest	0.0	Astrocytes TNFalpha + IL-1beta	0.0
Secondary CD8 lymphocyte act	0.0	KU-812 (Basophil) rest	1.6
CD4 lymphocyte none	0.0	KU-812 (Basophil) PMA/ionomycin	3.0
2ry Th1/Th2/Tr1_anti-CD95 CH11	0.0	CCD1106 (Keratinocytes) none	0.0
LAK cells rest	0.0	CCD1106 (Keratinocytes) TNFalpha + IL-1beta	0.0
LAK cells IL-2	0.0	Liver cirrhosis	12.9
LAK cells IL-2+IL-12	0.0	Lupus kidney	5.6
LAK cells IL-2+IFN gamma	0.2	NCI-H292 none	0.6
LAK cells IL-2+ IL-18	0.0	NCI-H292 IL-4	0.2
LAK cells PMA/ionomycin	0.0	NCI-H292 IL-9	0.0
NK Cells IL-2 rest	0.0	NCI-H292 IL-13	0.0
Two Way MLR 3 day	0.5	NCI-H292 IFN gamma	0.2
Two Way MLR 5 day	0.5	HPAEC none	0.4
Two Way MLR 7 day	0.0	HPAEC TNF alpha + IL-1 beta	0.2

PBMC rest	0.0	Lung fibroblast none	0.3
PBMC PWM	1.1	Lung fibroblast TNF alpha + IL-1 beta	0.0
PBMC PHA-L	0.6	Lung fibroblast IL-4	0.0
Ramos (B cell) none	0.0	Lung fibroblast IL-9	0.0
Ramos (B cell) ionomycin	0.0	Lung fibroblast IL-13	0.2
B lymphocytes PWM	0.0	Lung fibroblast IFN gamma	0.0
B lymphocytes CD40L and IL-4	0.0	Dermal fibroblast CCD1070 rest	0.0
EOL-1 dbcAMP	0.0	Dermal fibroblast CCD1070 TNF alpha	0.0
EOL-1 dbcAMP PMA/ionomycin	0.0	Dermal fibroblast CCD1070 IL-1 beta	0.0
Dendritic cells none	0.8	Dermal fibroblast IFN gamma	0.0
Dendritic cells LPS	4.6	Dermal fibroblast IL-4	0.0
Dendritic cells anti-CD40	0.4	IBD Colitis 2	0.0
Monocytes rest	0.0	IBD Crohn's	0.0
Monocytes LPS	21.8	Colon	3.8
Macrophages rest	0.2	Lung	3.2
Macrophages LPS	1.6	Thymus	**100.0**
HUVEC none	0.2	Kidney	0.2
HUVEC starved	0.3		

Panel 1.2 Summary: Ag1437 The expression of the CG56006-01 gene appears to be highest in a sample derived from normal adult kidney tissue (CT=23.3). In addition, there is substantial expression in samples derived from liver tissue. Of note is the difference in expression of this gene between adult and fetal kidney (CT=29) tissue. Thus, the expression of this gene could be used to distinguish normal kidney tissue from other samples in the panel, and in particular, from fetal kidney tissue.

There are also moderate to high levels of expression of this putative hepsin in a number of endocrine/metabolic related tissues including adrenal, GI tract, kidney, liver, pancreas and skeletal muscle and thyroid. Therefore, a therapeutic modulator to this gene and/or gene product may prove useful in the treatment of diseases where these tissues are involved.

Panel 1.3D Summary: Ag1437 The expression of the CG56006-01 gene was assessed in two independent runs on panel 1.3D with excellent concordance between runs. The expression of this gene appears to be highest in samples derived from liver tissue. In addition, there is substantial expression associated with normal kidney, bladder, stomach and pancreas tissue.

There is also substantial expression associated with cell lines derived from liver cancer, breast cancer and colon cancer. Thus, the expression of this gene could be used to distinguish liver derived samples from other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, protein therapeutics or antibodies may be

5   useful in the treatment of colon cancer, breast cancer or liver cancer.

In addition, this gene is expressed at much higher levels in fetal (CTs=33) when compared to adult skeletal muscle (CTs=36-40). This observation suggests that expression of this gene can be used to distinguish fetal from adult skeletal muscle. In addition, the relative overexpression of this gene in fetal skeletal muscle suggests that the protein product may enhance muscular

10   growth or development in the fetus and thus may also act in a regenerative capacity in the adult. Therefore, therapeutic modulation of the protein encoded by this gene could be useful in treatment of muscle related diseases. More specifically, treatment of weak or dystrophic muscle with the protein encoded by this gene could restore muscle mass or function.

Panel 2D Summary: Ag1437 The expression of the CG56006-01 gene was assessed in two

15   independent runs on panel 2D with excellent concordance between runs. The expression of this gene appears to be highest in samples derived from liver tissue, in particular malignant liver. This expression is consistent with the expression seen in Panel 1.3D. In addition, there is substantial expression associated with normal kidney tissues, when compared to their malignant counterparts, and breast and prostate cancers. Thus, the expression of this gene

20   could be used to distinguish liver derived samples from other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, protein therapeutics or antibodies may be useful in the treatment of liver cancer, kidney cancer, breast cancer or prostate cancer.

References:

25   Magee JA, Araki T, Patil S, Ehrig T, True L, Humphrey PA, Catalona WJ, Watson MA, Milbrandt J.Expression profiling reveals hepsin overexpression in prostate cancer. Cancer Res 2001 Aug 1;61(15):5692-6

Prostate cancer is the most commonly diagnosed noncutaneous cancer in men. Despite this fact, many of the genetic changes that coincide with prostate cancer progression remain

30   enigmatic. We have addressed this problem by characterizing the expression profiles of several benign and malignant human prostate samples, and we have identified several genes that are differentially expressed between benign and malignant glands. One gene that was overexpressed encodes the serine protease hepsin. We used an independent sample set to

confirm that hepsin is overexpressed in prostate tumors, and in situ hybridization demonstrates that hepsin is specifically overexpressed in the carcinoma cells themselves. These facts, together with the molecular properties of hepsin, make it an ideal target for prostate cancer therapy.

5   PMID: 11479199

Panel 4D Summary: Ag1437 The CG56006-01 transcript is expressed almost exclusively in activated monocytes at low but significant levels. This transcript encodes a serine protease hepsin,a transmembrane protease which has implicated in cell growth and maintenance. The expression of this transcript in LPS treated monocytes, cells that play a crucial role in linking

10   innate immunity to adaptive immunity, suggests a role for this gene product in initiating inflammatory reactions. Therefore, modulation of the expression or activity of this gene through the application of monoclonal antibodies may reduce or prevent early stages of inflammation and reduce the severity of inflammatory diseases such as psoriasis, asthma, inflammatory bowel disease, rheumatoid arthritis, osteoarthritis and other lung inflammatory

15   diseases.

NOV2 (CG56149-01)

Expression of gene CG56149-01 was assessed using the primer-probe sets Ag1672, Ag1673 and Ag3263, described in Tables 38A, 38B and 38C. Results of the RTQ-PCR runs are shown in Table 38D.

20

Table 38A. Probe Name Ag1672

Primers	Sequences	Length	Start Position
Forward	5'-gaccaaactttggccatttaa-3' (SEQ ID NO:210)	21	1568
Probe	TET-5'-cggatccatttgacacaccagcattt-3'-TAMRA (SEQ ID NO:211)	26	1589
Reverse	5'-gtgatggtcagagcatgaattt-3' (SEQ ID NO:212)	22	1646

Table 38B. Probe Name Ag1673

Primers	Sequences	Length	Start Position
Forward	5'-gaccaaactttggccatttaa-3' (SEQ ID NO:213)	21	1568
Probe	TET-5'-cggatccatttgacacaccagcattt-3'-TAMRA (SEQ ID NO:214)	26	1589
Reverse	5'-gtgatggtcagagcatgaattt-3' (SEQ ID NO:215)	22	1646

25   Table 38C. Probe Name Ag3263

Primers	Sequences	Length	Start Position
Forward	5'-tggggtagttggagctgaa-3' (SEQ ID NO:216)	19	876
Probe	TET-5'-caggtctgcacctgttcagcatttg-3'-TAMRA (SEQ ID NO:217)	25	897

| Reverse | 5'-tgcagacagaactgtgtcagtt-3' (SEQ ID NO:218) | 22 | 954 |

Table 38D. Panel 1.3D

Tissue Name	Rel. Exp.(%) Ag1672, Run 147227540	Rel. Exp.(%) Ag1673, Run 146581465	Tissue Name	Rel. Exp.(%) Ag1672, Run 147227540	Rel. Exp.(%) Ag1673, Run 146581465
Liver adenocarcinoma	41.8	36.6	Kidney (fetal)	12.2	14.9
Pancreas	5.3	7.4	Renal ca. 786-0	18.2	18.3
Pancreatic ca. CAPAN 2	9.5	9.1	Renal ca. A498	47.6	55.1
Adrenal gland	13.3	16.6	Renal ca. RXF 393	6.8	9.9
Thyroid	16.8	18.6	Renal ca. ACHN	37.1	41.2
Salivary gland	12.3	10.7	Renal ca. UO-31	37.1	39.0
Pituitary gland	24.5	31.6	Renal ca. TK-10	27.7	43.8
Brain (fetal)	8.2	9.7	Liver	0.0	5.6
Brain (whole)	25.7	26.8	Liver (fetal)	24.7	31.2
Brain (amygdala)	20.0	21.5	Liver ca. (hepatoblast) HepG2	27.9	31.6
Brain (cerebellum)	8.7	9.2	Lung	12.2	14.6
Brain (hippocampus)	41.2	37.4	Lung (fetal)	32.3	32.3
Brain (substantia nigra)	7.2	9.0	Lung ca. (small cell) LX-1	21.5	34.6
Brain (thalamus)	9.3	20.7	Lung ca. (small cell) NCI-H69	29.5	35.6
Cerebral Cortex	33.7	39.2	Lung ca. (s.cell var.) SHP-77	61.6	61.6
Spinal cord	15.5	19.2	Lung ca. (large cell)NCI-H460	29.9	33.9
glio/astro U87-MG	44.8	52.1	Lung ca. (non-sm. cell) A549	16.8	15.2
glio/astro U-118-MG	**100.0**	89.5	Lung ca. (non-s.cell) NCI-H23	79.0	92.7
astrocytoma SW1783	29.5	45.1	Lung ca. (non-s.cell) HOP-62	36.6	41.2

neuro*; met SK-N-AS	64.6	67.4	Lung ca. (non-s.cl) NCI-H522	30.8	37.9
astrocytoma SF-539	33.2	34.4	Lung ca. (squam.) SW 900	15.7	19.5
astrocytoma SNB-75	84.7	80.7	Lung ca. (squam.) NCI-H596	15.0	15.8
glioma SNB-19	30.1	43.2	Mammary gland	27.5	40.6
glioma U251	32.8	41.5	Breast ca.* (pl.ef) MCF-7	46.7	42.9
glioma SF-295	35.8	43.5	Breast ca.* (pl.ef) MDA-MB-231	84.7	86.5
Heart (fetal)	17.0	18.3	Breast ca.* (pl.ef) T47D	36.3	34.4
Heart	10.7	11.6	Breast ca. BT-549	94.0	80.1
Skeletal muscle (fetal)	49.0	44.4	Breast ca. MDA-N	27.7	29.5
Skeletal muscle	55.1	57.8	Ovary	9.6	11.2
Bone marrow	18.3	23.5	Ovarian ca. OVCAR-3	21.6	23.0
Thymus	21.3	21.0	Ovarian ca. OVCAR-4	9.3	9.2
Spleen	14.8	20.0	Ovarian ca. OVCAR-5	37.1	34.6
Lymph node	18.7	21.2	Ovarian ca. OVCAR-8	45.4	44.8
Colorectal	7.0	10.4	Ovarian ca. IGROV-1	13.2	16.4
Stomach	25.9	28.9	Ovarian ca.* (ascites) SK-OV-3	66.4	63.7
Small intestine	13.5	15.7	Uterus	17.3	18.6
Colon ca. SW480	52.5	50.0	Placenta	37.9	37.1
Colon ca.* SW620(SW480 met)	19.3	21.8	Prostate	9.3	11.5
Colon ca. HT29	21.9	33.2	Prostate ca.* (bone met)PC-3	23.8	35.8
Colon ca. HCT-116	35.4	29.9	Testis	87.1	84.1

Colon ca. CaCo-2	32.3	35.4	Melanoma Hs688(A).T	55.5	57.8
Colon ca. tissue(ODO3866)	25.7	29.3	Melanoma* (met) Hs688(B).T	74.7	88.9
Colon ca. HCC-2998	44.1	44.8	Melanoma UACC-62	3.0	3.8
Gastric ca.* (liver met) NCI-N87	95.3	**100.0**	Melanoma M14	7.4	11.9
Bladder	9.9	11.8	Melanoma LOX IMVI	3.3	4.4
Trachea	23.5	30.8	Melanoma* (met) SK-MEL-5	13.4	18.8
Kidney	5.6	3.8	Adipose	12.4	13.8

Panel 1.3D Summary: Ag1672/Ag1673 Two experiments with the same probe and primer set produce results that are in excellent agreement with highest expression of the CG56149-01 gene in a gastric cancer cell line (NCI-N87) or a brain cancer cell line (U-118-MG)(CTs=26-27). Thus, the expression of this gene could be used to distinguish these samples from other samples in the panel.

This gene encodes a protein that is homologous to nardilysin, an N-arginine (R) dibasic (NRD) convertase metalloendopeptidase of the M16 family, that specifically cleaves peptide substrates at the N-terminus of arginines in dibasic motifs in vitro. The peptidase M16 family is also known as the insulinase family and nardilysin is the closest homolog of the insulin degrading enzyme, insulinase. The ability of nardilysin to degrade insulin has not been proven. However, the high levels of expression in metabolic tissues in this panel, including adipose, fetal and adult skeletal muscle, pancreas, adrenal, thyroid and pituitary glands suggest that this gene product may have a profound effect on limiting the degradation of insulin in tissues relevant to type II diabetes (e.g. adipose, skeletal muscle).

There is also a significant level of difference between expression in adult(CTs=31-40) and fetal liver tissue(CTs=28), making this gene and/or gene-product a good candidate for distinguishing both forms. A putative role for this gene-product is in the post-translational processing of bioactive peptides from their inactive precursors.

This gene is also highly expressed in the testis. Nardilysis has been implicated in spermiogenesis. Thus, expression of this gene could be used as a marker for testis tissue. Furthermore, therapeutic modulation of the expression or function of this gene may be useful in the treatment of male reproductive disorders. A third experiment with the probe and primer

set Ag3263 shows low/undetectable levels of expression in all the samples on this panel. (CTs>35). (Data not shown.)

References:

Hospital V, Chesneau V, Balogh A, Joulie C, Seidah NG, Cohen P, Prat A. N-arginine dibasic convertase (nardilysin) isoforms are soluble dibasic-specific metalloendopeptidases that localize in the cytoplasm and at the cell surface. Biochem J 2000 Jul 15;349(Pt 2):587-97

N-arginine (R) dibasic (NRD) convertase (nardilysin; EC 3.4.24.61), a metalloendopeptidase of the M16 family, specifically cleaves peptide substrates at the N-terminus of arginines in dibasic motifs in vitro. In rat testis, the enzyme localizes within the cytoplasm of spermatids and associates with microtubules of the manchette and axoneme. NRD1 and NRD2 convertases, two NRD convertase isoforms, differ by the absence (isoform 1) or presence (isoform 2) of a 68-amino acid insertion close to the active site. In this study, we overexpressed both isoforms, either by vaccinia virus infection of BSC40 cells or transfection of COS-7 cells. The partially purified enzymes exhibit very similar biochemical and enzymic properties. Microsequencing revealed that NRD convertase is N-terminally processed. Results of immunocytofluorescence, immunoelectron microscopy and subcellular fractionation studies argue in favour of a primary cytosolic localization of both peptidases. Although the putative signal peptide did not direct NRD convertase into microsomes in an in vitro translation assay, biotinylation experiments clearly showed the presence of both isoforms at the cell surface. In conclusion, although most known processing events at pairs of basic residues are achieved by proprotein convertases within the secretory pathway, NRD convertase may fulfil a similar function in the cytoplasm and/or at the cell surface.

PMID: 10880358

Hospital V, Prat A, Joulie C, Cherif D, Day R, Cohen P. Human and rat testis express two mRNA species encoding variants of NRD convertase, a metalloendopeptidase of the insulinase family. Biochem J 1997 Nov 1;327 (Pt 3):773-9

Rat testis NRD convertase (EC 3.4.24.61) is a Zn2+dependent endopeptidase that cleaves, in vitro, peptide substrates at the N-terminus of Arg residues in dibasic sites. This putative

processing enzyme of the insulinase family of metallopeptidases exhibits a significant degree of similarity to insulinase and two yeast processing enzymes, Ax11 and Ste23. We report the cloning of two human testis cDNA species encoding isoforms of NRD convertase, hNRD1 and hNRD2. Whereas the hNRD1 transcript (3.7 kb) is equivalent to the previously

5   characterized rat cDNA (rNRD1), hNRD2 and rNRD2 are 3.9 kb novel forms containing a nucleotide insertion encoding a 68-residue segment. This motif, which is inserted N-terminal of the $Zn2+$binding site, HXXEH, is contained within the most conserved region among the insulinase family members. Analysis of the deduced primary sequences revealed 92% identity between rat and human orthologues. The human gene encoding NRD convertase was localized

10   to chromosome 1p32.1-p32.2. Whereas NRD convertase is mostly expressed in testis and in 24 cell lines, low mRNA levels were detected in most of the 27 other tissues tested.

PMID: 9581555

15   Chesneau V, Prat A, Segretain D, Hospital V, Dupaix A, Foulon T, Jegou B, Cohen P. NRD convertase: a putative processing endoprotease associated with the axoneme and the manchette in late spermatids.

J Cell Sci 1996 Nov;109 (Pt 11):2737-45

20

N-arginine dibasic convertase is a novel metalloendopeptidase which selectively cleaves at the N terminus of arginine residues in paired basic amino acids. Although present in brain and several other tissues, NRD convertase is particularly abundant in testis, where its expression appeared to be restricted to germ cells. Low levels of both mRNA and its corresponding

25   protein were detected early in spermatogenesis. However, a marked accumulation of the protein was observed during late steps (14 to 19) of spermiogenesis. By electron microscopy, the NRD convertase immunoreactivity was localized in the cytoplasm of elongating and elongated spermatids, with a noticeable concentration at the level of two microtubular structures, i.e. the manchette and the axoneme. These observations strongly support the

30   hypothesis that NRD convertase is involved in processing events potentially associated with the morphological transformations occurring during spermiogenesis.

PMID: 8937991

Panel 5D Summary: Ag3263 Expression of the CG56149-01 gene is low/undetectable in all samples on this panel (CTs>35). (Data not shown).

NOV5 (CG56151-01)

Expression of gene CG56151-01 was assessed using the primer-probe set Ag1681, described in Table 39A. Results of the RTQ-PCR runs are shown in Tables 39B, 39C, 39D and 39E.

Table 39A. Probe Name Ag1681

Primers	Sequences	Length	Start Position
Forward	5'-ggacttctgtggaccttatgtg-3' (SEQ ID NO:219)	22	1412
Probe	TET-5'-ttttcctctttgctggagtgctcctg-3'-TAMRA (SEQ ID NO:220)	26	1435
Reverse	5'-ttcctttggtttctggaacttt-3' (SEQ ID NO:221)	22	1485

Table 39B. General_screening_panel_v1.4

Tissue Name	Rel. Exp.(%) Ag1681, Run 208016706	Tissue Name	Rel. Exp.(%) Ag1681, Run 208016706
Adipose	0.0	Renal ca. TK-10	0.1
Melanoma* Hs688(A).T	0.0	Bladder	0.4
Melanoma* Hs688(B).T	0.0	Gastric ca. (liver met.) NCI-N87	0.7
Melanoma* M14	0.2	Gastric ca. KATO III	0.1
Melanoma* LOXIMVI	0.2	Colon ca. SW-948	0.0
Melanoma* SK-MEL-5	0.0	Colon ca. SW480	0.2
Squamous cell carcinoma SCC-4	0.0	Colon ca.* (SW480 met) SW620	0.0
Testis Pool	0.4	Colon ca. HT29	0.1
Prostate ca.* (bone met) PC-3	0.0	Colon ca. HCT-116	0.1
Prostate Pool	0.0	Colon ca. CaCo-2	3.7
Placenta	0.0	Colon cancer tissue	0.0
Uterus Pool	0.0	Colon ca. SW1116	0.0
Ovarian ca. OVCAR-3	0.4	Colon ca. Colo-205	0.1
Ovarian ca. SK-OV-3	0.0	Colon ca. SW-48	0.0
Ovarian ca. OVCAR-4	0.0	Colon Pool	0.0
Ovarian ca. OVCAR-5	0.1	Small Intestine Pool	0.0

Ovarian ca. IGROV-1	0.0	Stomach Pool	0.2
Ovarian ca. OVCAR-8	0.1	Bone Marrow Pool	0.0
Ovary	0.2	Fetal Heart	0.0
Breast ca. MCF-7	0.2	Heart Pool	0.1
Breast ca. MDA-MB-231	0.0	Lymph Node Pool	0.1
Breast ca. BT 549	0.0	Fetal Skeletal Muscle	0.1
Breast ca. T47D	0.1	Skeletal Muscle Pool	0.0
Breast ca. MDA-N	0.2	Spleen Pool	0.1
Breast Pool	0.1	Thymus Pool	0.0
Trachea	0.0	CNS cancer (glio/astro) U87-MG	0.0
Lung	0.1	CNS cancer (glio/astro) U-118-MG	0.0
Fetal Lung	1.2	CNS cancer (neuro;met) SK-N-AS	0.0
Lung ca. NCI-N417	0.0	CNS cancer (astro) SF-539	0.0
Lung ca. LX-1	0.1	CNS cancer (astro) SNB-75	0.1
Lung ca. NCI-H146	0.0	CNS cancer (glio) SNB-19	0.0
Lung ca. SHP-77	0.0	CNS cancer (glio) SF-295	0.0
Lung ca. A549	0.0	Brain (Amygdala) Pool	0.2
Lung ca. NCI-H526	0.0	Brain (cerebellum)	0.0
Lung ca. NCI-H23	0.2	Brain (fetal)	0.0
Lung ca. NCI-H460	0.0	Brain (Hippocampus) Pool	0.1
Lung ca. HOP-62	0.6	Cerebral Cortex Pool	0.2
Lung ca. NCI-H522	0.0	Brain (Substantia nigra) Pool	0.4
Liver	23.2	Brain (Thalamus) Pool	0.1
Fetal Liver	**100.0**	Brain (whole)	0.4
Liver ca. HepG2	7.4	Spinal Cord Pool	0.3
Kidney Pool	0.1	Adrenal Gland	0.1
Fetal Kidney	1.9	Pituitary gland Pool	0.1
Renal ca. 786-0	0.6	Salivary Gland	0.0
Renal ca. A498	0.1	Thyroid (female)	0.0
Renal ca. ACHN	4.8	Pancreatic ca. CAPAN2	0.5
Renal ca. UO-31	0.1	Pancreas Pool	0.5

Table 39C. Panel 1.3D

Tissue Name	Rel. Exp.(%) Ag1681, Run 146581527	Tissue Name	Rel. Exp.(%) Ag1681, Run 146581527
Liver adenocarcinoma	0.0	Kidney (fetal)	5.8
Pancreas	1.5	Renal ca. 786-0	0.5
Pancreatic ca. CAPAN 2	0.3	Renal ca. A498	0.5
Adrenal gland	0.1	Renal ca. RXF 393	0.1
Thyroid	0.0	Renal ca. ACHN	11.7
Salivary gland	0.0	Renal ca. UO-31	0.2
Pituitary gland	0.2	Renal ca. TK-10	0.1
Brain (fetal)	0.0	Liver	**100.0**
Brain (whole)	0.0	Liver (fetal)	99.3
Brain (amygdala)	0.0	Liver ca. (hepatoblast) HepG2	22.2
Brain (cerebellum)	0.0	Lung	0.0
Brain (hippocampus)	0.1	Lung (fetal)	0.0
Brain (substantia nigra)	0.0	Lung ca. (small cell) LX-1	0.0
Brain (thalamus)	0.0	Lung ca. (small cell) NCI-H69	0.0
Cerebral Cortex	0.1	Lung ca. (s.cell var.) SHP-77	0.0
Spinal cord	0.1	Lung ca. (large cell)NCI-H460	0.0
glio/astro U87-MG	0.2	Lung ca. (non-sm. cell) A549	0.1
glio/astro U-118-MG	0.0	Lung ca. (non-s.cell) NCI-H23	0.7
astrocytoma SW1783	0.2	Lung ca. (non-s.cell) HOP-62	0.5
neuro*; met SK-N-AS	0.1	Lung ca. (non-s.cl) NCI-H522	0.0
astrocytoma SF-539	0.2	Lung ca. (squam.) SW 900	0.1
astrocytoma SNB-75	0.0	Lung ca. (squam.) NCI-H596	0.0
glioma SNB-19	0.1	Mammary gland	0.0
glioma U251	0.0	Breast ca.* (pl.ef) MCF-7	0.2
glioma SF-295	0.0	Breast ca.* (pl.ef) MDA-MB-231	0.2
Heart (fetal)	0.0	Breast ca.* (pl.ef) T47D	0.2
Heart	0.0	Breast ca. BT-549	0.0
Skeletal muscle (fetal)	0.0	Breast ca. MDA-N	0.4

Skeletal muscle	0.0	Ovary	0.1
Bone marrow	0.0	Ovarian ca. OVCAR-3	0.3
Thymus	0.0	Ovarian ca. OVCAR-4	0.1
Spleen	0.0	Ovarian ca. OVCAR-5	0.2
Lymph node	0.0	Ovarian ca. OVCAR-8	0.2
Colorectal	0.3	Ovarian ca. IGROV-1	0.0
Stomach	0.1	Ovarian ca.* (ascites) SK-OV-3	0.0
Small intestine	7.6	Uterus	0.0
Colon ca. SW480	0.3	Placenta	0.0
Colon ca.* SW620(SW480 met)	0.0	Prostate	0.0
Colon ca. HT29	0.2	Prostate ca.* (bone met)PC-3	0.0
Colon ca. HCT-116	0.0	Testis	0.2
Colon ca. CaCo-2	8.8	Melanoma Hs688(A).T	0.0
Colon ca. tissue(ODO3866)	0.1	Melanoma* (met) Hs688(B).T	0.1
Colon ca. HCC-2998	1.7	Melanoma UACC-62	0.0
Gastric ca.* (liver met) NCI-N87	0.8	Melanoma M14	0.2
Bladder	0.4	Melanoma LOX IMVI	0.0
Trachea	0.0	Melanoma* (met) SK-MEL-5	0.0
Kidney	8.9	Adipose	0.1

Table 39D. Panel 2D

Tissue Name	Rel. Exp.(%) Ag1681, Run 148168295	Tissue Name	Rel. Exp.(%) Ag1681, Run 148168295
Normal Colon	0.5	Kidney Margin 8120608	1.7
CC Well to Mod Diff (ODO3866)	0.0	Kidney Cancer 8120613	0.0
CC Margin (ODO3866)	0.0	Kidney Margin 8120614	2.2
CC Gr.2 rectosigmoid (ODO3868)	0.0	Kidney Cancer 9010320	0.1
CC Margin (ODO3868)	0.0	Kidney Margin	4.0

		9010321	
CC Mod Diff (ODO3920)	0.1	Normal Uterus	0.0
CC Margin (ODO3920)	0.0	Uterus Cancer 064011	0.0
CC Gr.2 ascend colon (ODO3921)	0.0	Normal Thyroid	0.1
CC Margin (ODO3921)	0.0	Thyroid Cancer 064010	0.2
CC from Partial Hepatectomy (ODO4309) Mets	6.6	Thyroid Cancer A302152	0.1
Liver Margin (ODO4309)	**100.0**	Thyroid Margin A302153	0.1
Colon mets to lung (OD04451-01)	0.0	Normal Breast	0.1
Lung Margin (OD04451-02)	0.0	Breast Cancer (OD04566)	0.1
Normal Prostate 6546-1	0.0	Breast Cancer (OD04590-01)	0.0
Prostate Cancer (OD04410)	0.0	Breast Cancer Mets (OD04590-03)	0.1
Prostate Margin (OD04410)	0.0	Breast Cancer Metastasis (OD04655-05)	0.0
Prostate Cancer (OD04720-01)	0.0	Breast Cancer 064006	0.5
Prostate Margin (OD04720-02)	0.0	Breast Cancer 1024	0.0
Normal Lung 061010	0.1	Breast Cancer 9100266	0.0
Lung Met to Muscle (ODO4286)	0.0	Breast Margin 9100265	0.0
Muscle Margin (ODO4286)	0.0	Breast Cancer A209073	0.1
Lung Malignant Cancer (OD03126)	0.0	Breast Margin A2090734	0.1
Lung Margin (OD03126)	0.0	Normal Liver	86.5
Lung Cancer (OD04404)	0.0	Liver Cancer 064003	23.5
Lung Margin (OD04404)	0.0	Liver Cancer 1025	39.8
Lung Cancer (OD04565)	0.0	Liver Cancer 1026	13.6
Lung Margin (OD04565)	0.0	Liver Cancer 6004-T	47.0
Lung Cancer (OD04237-01)	0.0	Liver Tissue 6004-N	8.1
Lung Margin (OD04237-02)	0.0	Liver Cancer 6005-T	12.6
Ocular Mel Met to Liver	0.0	Liver Tissue 6005-N	14.1

(ODO4310)			
Liver Margin (ODO4310)	62.0	Normal Bladder	0.3
Melanoma Mets to Lung (OD04321)	0.0	Bladder Cancer 1023	0.0
Lung Margin (OD04321)	0.0	Bladder Cancer A302173	0.0
Normal Kidney	9.2	Bladder Cancer (OD04718-01)	0.0
Kidney Ca, Nuclear grade 2 (OD04338)	1.0	Bladder Normal Adjacent (OD04718-03)	0.0
Kidney Margin (OD04338)	1.5	Normal Ovary	0.0
Kidney Ca Nuclear grade 1/2 (OD04339)	0.0	Ovarian Cancer 064008	0.0
Kidney Margin (OD04339)	12.9	Ovarian Cancer (OD04768-07)	0.8
Kidney Ca, Clear cell type (OD04340)	10.4	Ovary Margin (OD04768-08)	0.0
Kidney Margin (OD04340)	3.7	Normal Stomach	0.0
Kidney Ca, Nuclear grade 3 (OD04348)	0.3	Gastric Cancer 9060358	0.0
Kidney Margin (OD04348)	2.1	Stomach Margin 9060359	0.0
Kidney Cancer (OD04622-01)	1.0	Gastric Cancer 9060395	0.0
Kidney Margin (OD04622-03)	0.5	Stomach Margin 9060394	0.3
Kidney Cancer (OD04450-01)	0.4	Gastric Cancer 9060397	0.0
Kidney Margin (OD04450-03)	2.2	Stomach Margin 9060396	0.0
Kidney Cancer 8120607	0.0	Gastric Cancer 064005	0.4

Table 39E. Panel 5D

Tissue Name	Rel. Exp.(%) Ag1681, Run 169271477	Tissue Name	Rel. Exp.(%) Ag1681, Run 169271477
97457_Patient-02go_adipose	0.2	94709_Donor 2 AM - A_adipose	0.0
97476_Patient-07sk_skeletal muscle	0.0	94710_Donor 2 AM - B_adipose	0.0
97477_Patient-07ut_uterus	0.0	94711_Donor 2 AM - C_adipose	0.0

97478_Patient-07pl_placenta	0.0	94712_Donor 2 AD - A_adipose	•0.0
97481_Patient-08sk_skeletal muscle	0.0	94713_Donor 2 AD - B_adipose	0.0
97482_Patient-08ut_uterus	0.0	94714_Donor 2 AD - C_adipose	0.0
97483_Patient-08pl_placenta	0.2	94742_Donor 3 U - A_Mesenchymal Stem Cells	0.0
97486_Patient-09sk_skeletal muscle	0.0	94743_Donor 3 U - B_Mesenchymal Stem Cells	0.0
97487_Patient-09ut_uterus	0.1	94730_Donor 3 AM - A_adipose	0.0
97488_Patient-09pl_placenta	0.0	94731_Donor 3 AM - B_adipose	0.0
97492_Patient-10ut_uterus	0.0	94732_Donor 3 AM - C_adipose	0.0
97493_Patient-10pl_placenta	0.0	94733_Donor 3 AD - A_adipose	0.0
97495_Patient-11go_adipose	0.0	94734_Donor 3 AD - B_adipose	0.0
97496_Patient-11sk_skeletal muscle	0.0	94735_Donor 3 AD - C_adipose	0.0
97497_Patient-11ut_uterus	0.0	77138_Liver_HepG2untreated	28.3
97498_Patient-11pl_placenta	0.0	73556_Heart_Cardiac stromal cells (primary)	0.0
97500_Patient-12go_adipose	0.3	81735_Small Intestine	12.3
97501_Patient-12sk_skeletal muscle	0.0	72409_Kidney_Proximal Convoluted Tubule	4.3
97502_Patient-12ut_uterus	0.2	82685_Small intestine_Duodenum	**100.0**
97503_Patient-12pl_placenta	0.0	90650_Adrenal_Adrenocortical adenoma	0.0
94721_Donor 2 U - A_Mesenchymal Stem Cells	0.0	72410_Kidney_HRCE	3.6
94722_Donor 2 U - B_Mesenchymal Stem Cells	0.0	72411_Kidney_HRE	0.1
94723_Donor 2 U - C_Mesenchymal Stem Cells	0.0	73139_Uterus_Uterine smooth muscle cells	0.0

General_screening_panel_v1.4 Summary: Ag1681 The CG56151-01 gene, a glucose transporter type 2 homolog, is predominantly expressed in liver. GLUT2 facilitates the

transport of glucose into the liver. This gene is also expressed in brain, pancreas, and testis. This is consistent with immunocytochemistry data that shows that the Glut2 gene is expressed in insulin producing beta cells in the pancreas and aids in regulation of insulin secretion. Since the liver is responsible for gluconeogenesis, enhancing glucose uptake through GLUT2 may

5   produce a negative feedback loop that would decrease hepatic glucose production. This could result in a lowering of blood glucose, a major therapeutic goal for the treatment of Type II (non-insulin dependent) diabetes. Thus, enhancing the function of the protein encoded by the CG56151-01 gene with an agonist antibody therapeutic could restore balance to blood glucose levels in patients with Type II diabetes.

10   In addition, this gene is expressed at higher levels in fetal liver and lung (CTs=29) than in the adult sources of these tissues. Thus, expression of this gene could be used to differentiate between the two sources of these tissues.

References:

Waeber G, Pedrazzini T, Bonny O, Bonny C, Steinmann M, Nicod P, Haefliger JA. A 338-bp

15   proximal fragment of the glucose transporter type 2 (GLUT2) promoter drives reporter gene expression in the pancreatic islets of transgenic mice. Mol Cell Endocrinol 1995 Oct 30;114(1-2):205-15

The high Km glucose transporter GLUT2 is a membrane protein expressed in tissues involved in maintaining glucose homeostasis, and in cells where glucose-sensing is necessary. In many

20   experimental models of diabetes, GLUT2 gene expression is decreased in pancreatic beta-cells, which could lead to a loss of glucose-induced insulin secretion. In order to identify factors involved in pancreatic beta-cell specific expression of GLUT2, we have recently cloned the murine GLUT2 promoter and identified cis-elements within the 338-bp of the proximal promoter capable of binding islet-specific trans-acting factors. Furthermore, in

25   transient transfection studies, this 338-bp fragment could efficiently drive the expression of the chloramphenicol acetyl transferase (CAT) gene in cell lines derived from the endocrine pancreas, but displayed no promoter activity in non-pancreatic cells. In this report, we tested the cell-specific expression of a CAT reporter gene driven by a short (338 bp) and a larger (1311 bp) fragment of the GLUT2 promoter in transgenic mice. We generated ten transgenic

30   lines that integrated one of the constructs. CAT mRNA expression in transgenic tissues was assessed using the RNAse protection assay and the quantitative reverse transcribed polymerase chain reaction (RT-PCR). Overall CAT mRNA expression for both constructs was low compared to endogenous GLUT2 mRNA levels but the reporter transcript could be detected in

all animals in the pancreatic islets and the liver, and in a few transgenic lines in the kidney and the small intestine. The CAT protein was also present in Langerhans islets and in the liver for both constructs by immunocytochemistry. These findings suggest that the proximal 338 bp of the murine GLUT2 promoter contain cis-elements required for the islet-specific expression of

5   GLUT2.

PMID: 8674846

Panel 1.3D Summary: Ag1681 Expression of the CG56151-01 gene is restricted to liver derived tissue, an important metabolic tissue, in this panel (CTs=27). This liver specific expression is consistent with expression in other panels and with published data (see reference

10   below.) Thus, expression of this gene could be used as a marker for liver tissue. This gene encodes a glut2 homolog. Please see General_screening_panel_v1.4 for discussion of utility of this gene in metabolic disease.

References:

Rencurel F, Waeber G, Antoine B, Rocchiccioli F, Maulard P, Girard J, Leturque A.

15   Requirement of glucose metabolism for regulation of glucose transporter type 2 (GLUT2) gene expression in liver. Biochem J 1996 Mar 15;314 (Pt 3):903-9

Previous studies have shown that glucose increases the glucose transporter (GLUT2) mRNA expression in the liver in vivo and in vitro. Here we report an analysis of the effects of glucose metabolism on GLUT2 gene expression. GLUT2 mRNA accumulation by glucose was not due

20   to stabilization of its transcript but rather was a direct effect on gene transcription. A proximal fragment of the 5' regulatory region of the mouse GLUT2 gene linked to a reporter gene was transiently transfected into liver GLUT2-expressing cells. Glucose stimulated reporter gene expression in these cells, suggesting that glucose-responsive elements were included within the proximal region of the promoter. A dose-dependent effect of glucose on GLUT2

25   expression was observed over 10 mM glucose irrespective of the hexokinase isozyme (glucokinase K(m) 16 mM; hexokinase I K(m) 0.01 mM) present in the cell type used. This suggests that the correlation between extracellular glucose and GLUT2 mRNA concentrations is simply a reflection of an activation of glucose metabolism. The mediators and the mechanism responsible for this response remain to be determined. In conclusion, glucose

30   metabolism is required for the proper induction of the GLUT2 gene in the liver and this effect is transcriptionally regulated.

PMID: 8615787

Panel 2D Summary: Ag1681 The expression of the CG56151-01 gene appears to be highest in a sample of normal liver tissue adjacent to a colon cancer metastasis (CT=24.6). In addition, there is substantial expression in both normal and malignant liver tissue. This restricted pattern of expression in liver derived tissue is consistent with expression in the previous panels. Thus,

5   the expression of this gene could be used to distinguish liver derived tissue from other samples in the panel. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, protein therapeutics or antibodies might be beneficial in the treatment of liver cancer.

Panel 5D Summary: Ag1681 The expression pattern of the CG56151-01 gene, a Glut2 homolog, is limited to a liver cell line (HepG2) and small intestines. The presence of this

10   isoform in the intestines may indicate an important role in glucose uptake from the digestive tract. Please refer to panel 1.4 for a further discussion of utility of this gene in metabolic disease.

NOV3 (CG56155-01)

Expression of gene CG56155-01 was assessed using the primer-probe set Ag1688, described

15   in Table 40A. Results of the RTQ-PCR runs are shown in Tables 40B, 40C and 40D.

Table 40A. Probe Name Ag1688

Primers	Sequences	Length	Start Position
Forward	5'-tcagaagggaatcatgatatcg-3' (SEQ ID NO:222)	22	1503
Probe	TET-5'-ccttgataaaactccaggctcctttga-3'-TAMRA (SEQ ID NO:223)	27	1525
Reverse	5'-tttggaaggtaggcatattgg-3' (SEQ ID NO:224)	21	1572

Table 40B. Panel 1.3D

Tissue Name	Rel. Exp.(%) Ag1688, Run 147249266	Tissue Name	Rel. Exp.(%) Ag1688, Run 147249266
Liver adenocarcinoma	0.0	Kidney (fetal)	9.2
Pancreas	6.7	Renal ca. 786-0	0.0
Pancreatic ca. CAPAN 2	0.2	Renal ca. A498	1.7
Adrenal gland	1.8	Renal ca. RXF 393	0.0
Thyroid	3.8	Renal ca. ACHN	0.0
Salivary gland	1.5	Renal ca. UO-31	0.0
Pituitary gland	6.1	Renal ca. TK-10	0.0
Brain (fetal)	0.5	Liver	**100.0**
Brain (whole)	3.6	Liver (fetal)	99.3
Brain (amygdala)	3.3	Liver ca. (hepatoblast) HepG2	0.0
Brain (cerebellum)	0.4	Lung	1.3

Brain (hippocampus)	6.2	Lung (fetal)	1.8
Brain (substantia nigra)	1.0	Lung ca. (small cell) LX-1	0.0
Brain (thalamus)	2.1	Lung ca. (small cell) NCI-H69	0.0
Cerebral Cortex	6.3	Lung ca. (s.cell var.) SHP-77	0.8
Spinal cord	3.1	Lung ca. (large cell)NCI-H460	0.0
glio/astro U87-MG	0.0	Lung ca. (non-sm. cell) A549	0.2
glio/astro U-118-MG	0.0	Lung ca. (non-s.cell) NCI-H23	0.0
astrocytoma SW1783	0.0	Lung ca. (non-s.cell) HOP-62	0.0
neuro*; met SK-N-AS	0.2	Lung ca. (non-s.cl) NCI-H522	0.0
astrocytoma SF-539	0.0	Lung ca. (squam.) SW 900	0.2
astrocytoma SNB-75	0.1	Lung ca. (squam.) NCI-H596	0.0
glioma SNB-19	0.2	Mammary gland	2.9
glioma U251	1.2	Breast ca.* (pl.ef) MCF-7	0.0
glioma SF-295	0.0	Breast ca.* (pl.ef) MDA-MB-231	0.0
Heart (fetal)	0.2	Breast ca.* (pl.ef) T47D	0.0
Heart	1.6	Breast ca. BT-549	0.0
Skeletal muscle (fetal)	0.7	Breast ca. MDA-N	0.0
Skeletal muscle	1.2	Ovary	0.0
Bone marrow	0.5	Ovarian ca. OVCAR-3	0.2
Thymus	3.2	Ovarian ca. OVCAR-4	0.0
Spleen	1.0	Ovarian ca. OVCAR-5	0.3
Lymph node	2.9	Ovarian ca. OVCAR-8	0.0
Colorectal	0.8	Ovarian ca. IGROV-1	0.0
Stomach	3.3	Ovarian ca.* (ascites) SK-OV-3	1.0
Small intestine	6.2	Uterus	1.4
Colon ca. SW480	0.0	Placenta	0.4

Colon ca.* SW620(SW480 met)	0.0	Prostate	1.0
Colon ca. HT29	0.0	Prostate ca.* (bone met)PC-3	0.0
Colon ca. HCT-116	0.0	Testis	6.1
Colon ca. CaCo-2	0.2	Melanoma Hs688(A).T	0.4
Colon ca. tissue(ODO3866)	0.0	Melanoma* (met) Hs688(B).T	0.9
Colon ca. HCC-2998	0.2	Melanoma UACC-62	0.0
Gastric ca.* (liver met) NCI-N87	4.4	Melanoma M14	0.0
Bladder	3.1	Melanoma LOX IMVI	0.0
Trachea	3.0	Melanoma* (met) SK-MEL-5	0.0
Kidney	6.8	Adipose	0.5

<u>Table 40C</u>. Panel 2D

Tissue Name	Rel. Exp.(%) Ag1688, Run 162646059	Tissue Name	Rel. Exp.(%) Ag1688, Run 162646059
Normal Colon	1.7	Kidney Margin 8120608	0.7
CC Well to Mod Diff (ODO3866)	0.0	Kidney Cancer 8120613	0.0
CC Margin (ODO3866)	0.2	Kidney Margin 8120614	0.5
CC Gr.2 rectosigmoid (ODO3868)	0.2	Kidney Cancer 9010320	0.2
CC Margin (ODO3868)	0.1	Kidney Margin 9010321	1.0
CC Mod Diff (ODO3920)	0.1	Normal Uterus	0.2
CC Margin (ODO3920)	0.9	Uterus Cancer 064011	0.8
CC Gr.2 ascend colon (ODO3921)	0.1	Normal Thyroid	0.9
CC Margin (ODO3921)	0.1	Thyroid Cancer 064010	0.2
CC from Partial Hepatectomy (ODO4309) Mets	4.7	Thyroid Cancer A302152	0.5
Liver Margin (ODO4309)	**100.0**	Thyroid Margin A302153	1.0
Colon mets to lung (OD04451-01)	0.1	Normal Breast	0.3
Lung Margin (OD04451-	0.1	Breast Cancer	0.1

02)		(OD04566)	
Normal Prostate 6546-1	2.1	Breast Cancer (OD04590-01)	0.1
Prostate Cancer (OD04410)	0.6	Breast Cancer Mets (OD04590-03)	0.4
Prostate Margin (OD04410)	0.5	Breast Cancer Metastasis (OD04655-05)	0.9
Prostate Cancer (OD04720-01)	1.1	Breast Cancer 064006	0.6
Prostate Margin (OD04720-02)	1.6	Breast Cancer 1024	1.2
Normal Lung 061010	2.0	Breast Cancer 9100266	0.1
Lung Met to Muscle (ODO4286)	0.0	Breast Margin 9100265	0.1
Muscle Margin (ODO4286)	0.2	Breast Cancer A209073	0.3
Lung Malignant Cancer (OD03126)	0.1	Breast Margin A2090734	0.3
Lung Margin (OD03126)	0.5	Normal Liver	69.7
Lung Cancer (OD04404)	0.1	Liver Cancer 064003	13.7
Lung Margin (OD04404)	0.2	Liver Cancer 1025	18.0
Lung Cancer (OD04565)	0.0	Liver Cancer 1026	1.2
Lung Margin (OD04565)	0.1	Liver Cancer 6004-T	22.2
Lung Cancer (OD04237-01)	0.1	Liver Tissue 6004-N	1.0
Lung Margin (OD04237-02)	0.4	Liver Cancer 6005-T	1.9
Ocular Mel Met to Liver (ODO4310)	0.1	Liver Tissue 6005-N	4.2
Liver Margin (ODO4310)	77.4	Normal Bladder	2.7
Melanoma Mets to Lung (OD04321)	0.0	Bladder Cancer 1023	0.0
Lung Margin (OD04321)	0.1	Bladder Cancer A302173	0.2
Normal Kidney	12.9	Bladder Cancer (OD04718-01)	0.1
Kidney Ca, Nuclear grade 2 (OD04338)	3.8	Bladder Normal Adjacent (OD04718-03)	0.5
Kidney Margin (OD04338)	1.6	Normal Ovary	0.0
Kidney Ca Nuclear grade 1/2 (OD04339)	2.8	Ovarian Cancer 064008	0.1

Kidney Margin (OD04339)	9.3	Ovarian Cancer (OD04768-07)	0.2
Kidney Ca, Clear cell type (OD04340)	1.4	Ovary Margin (OD04768-08)	0.1
Kidney Margin (OD04340)	4.1	Normal Stomach	0.3
Kidney Ca, Nuclear grade 3 (OD04348)	0.1	Gastric Cancer 9060358	0.1
Kidney Margin (OD04348)	3.8	Stomach Margin 9060359	0.0
Kidney Cancer (OD04622-01)	0.2	Gastric Cancer 9060395	0.2
Kidney Margin (OD04622-03)	0.7	Stomach Margin 9060394	0.3
Kidney Cancer (OD04450-01)	0.2	Gastric Cancer 9060397	0.3
Kidney Margin (OD04450-03)	2.6	Stomach Margin 9060396	0.0
Kidney Cancer 8120607	0.0	Gastric Cancer 064005	1.1

Table 40D. Panel 5 Islet

Tissue Name	Rel. Exp.(%) Ag1688, Run 226587524	Tissue Name	Rel. Exp.(%) Ag1688, Run 226587524
97457_Patient-02go_adipose	41.2	94709_Donor 2 AM - A_adipose	0.0
97476_Patient-07sk_skeletal muscle	9.9	94710_Donor 2 AM - B_adipose	0.0
97477_Patient-07ut_uterus	8.1	94711_Donor 2 AM - C_adipose	0.0
97478_Patient-07pl_placenta	0.0	94712_Donor 2 AD - A_adipose	11.4
99167_Bayer Patient 1	84.7	94713_Donor 2 AD - B_adipose	0.0
97482_Patient-08ut_uterus	2.4	94714_Donor 2 AD - C_adipose	29.1
97483_Patient-08pl_placenta	0.0	94742_Donor 3 U - A_Mesenchymal Stem Cells	19.2
97486_Patient-09sk_skeletal muscle	8.0	94743_Donor 3 U - B_Mesenchymal Stem Cells	0.0
97487_Patient-09ut_uterus	9.6	94730_Donor 3 AM - A_adipose	15.0
97488_Patient-09pl_placenta	0.0	94731_Donor 3 AM - B_adipose	37.9
97492_Patient-10ut_uterus	0.0	94732_Donor 3 AM - C_adipose	0.0

97493_Patient-10pl_placenta	0.0	94733_Donor 3 AD - A_adipose	39.2
97495_Patient-11go_adipose	0.0	94734_Donor 3 AD - B_adipose	11.4
97496_Patient-11sk_skeletal muscle	52.9	94735_Donor 3 AD - C_adipose	34.4
97497_Patient-11ut_uterus	35.8	77138_Liver_HepG2untreated	8.4
97498_Patient-11pl_placenta	10.5	73556_Heart_Cardiac stromal cells (primary)	0.0
97500_Patient-12go_adipose	0.0	81735_Small Intestine	**100.0**
97501_Patient-12sk_skeletal muscle	35.4	72409_Kidney_Proximal Convoluted Tubule	9.9
97502_Patient-12ut_uterus	20.7	82685_Small intestine_Duodenum	70.2
97503_Patient-12pl_placenta	0.0	90650_Adrenal_Adrenocortical adenoma	25.5
94721_Donor 2 U - A_Mesenchymal Stem Cells	0.0	72410_Kidney_HRCE	10.4
94722_Donor 2 U - B_Mesenchymal Stem Cells	0.0	72411_Kidney_HRE	7.2
94723_Donor 2 U - C_Mesenchymal Stem Cells	0.0	73139_Uterus_Uterine smooth muscle cells	0.0

Panel 1.3D Summary: Ag1688 Expression of the CG56155-01 gene, a plasma kallikrein homolog, is significantly higher in liver (CTs=28) than in any other sample on this panel. Thus, expression of this gene could be used as a marker of liver tissue. Plasma kallikrein is a serine protease that, among other roles, plays a part in blood coagulation, fibrinolysis, and complement activation and has been implicated in adipose differentiation by remodelling of the fibronectin-rich ECM of preadipocytes. Therefore, an antagonist to this gene product may be beneficial in the treatment of obesity.

References:

Hoover-Plow J, Yuen L. Plasminogen binding is increased with adipocyte differentiation. Biochem.Biophys.Res.Commun. (2001) 284, 389-394

The purpose of this study was to examine the role of the plasminogen system in the development of adipose tissue. Plasminogen binding capacity was determined in differentiated and undifferentiated cells from adipose tissue of plasminogen deficient mice and 3T3 cells, a

well-characterized tissue culture model. In 3T3 cells, plasminogen binding was fivefold higher in differentiated cells compared to the undifferentiated cells. Inhibition of binding by carboxyl-terminal lysine analogs was similar for the differentiated and undifferentiated cells with tranexamic acid > EACA > lysine. The binding of plasminogen was concentration-dependent and approaches saturation in the both cell types. The number of plasminogen binding sites was tenfold higher in the differentiated compared to the undifferentiated cells. In isolated mature fat cells and stromal cell cultures from mouse adipose tissue, plasminogen binding was also higher in the differentiated mature fat cells and differentiated stromal cells compared to undifferentiated stromal cells. Plasminogen binding was elevated in the differentiated cells from the Plg-/- mice compared to cells from the WT mice. These results suggest that the plasminogen system plays an important role in adipose tissue development. Copyright 2001 Academic Press.

PMID: 11394891

Selvarajan S, Lund LR, Takeuchi T, Craik CS, Werb Z.A plasma kallikrein-dependent plasminogen cascade required for adipocyte differentiation. Nature Cell Biol. (2001) 3, 267-275.

Here we show that plasma kallikrein (PKal) mediates a plasminogen (Plg) cascade in adipocyte differentiation. Ecotin, an inhibitor of serine proteases, inhibits cell-shape change, adipocyte-specific gene expression, and lipid accumulation during adipogenesis in culture. Deficiency of Plg, but not of urokinase or tissue-type plasminogen activator, suppresses adipogenesis during differentiation of 3T3-L1 cells and mammary-gland involution. PKal, which is inhibited by ecotin, is required for adipose conversion, Plg activation and 3T3-L1 differentiation. Human plasma lacking PKal does not support differentiation of 3T3-L1 cells. PKal is therefore a physiological regulator that acts in the Plg cascade during adipogenesis. We propose that the Plg cascade fosters adipocyte differentiation by degradation of the fibronectin-rich preadipocyte stromal matrix.

PMID: 11231576

Panel 2D Summary: Ag1688 The expression of the CG56155-01 gene appears to be highest in a sample derived from a sample of normal liver tissue adjacent to a metastatic colon cancer CT=26.2). In addition, there is substantial expression in other samples of normal liver, and to a much lesser degree, malignant liver tissue. This liver specific expression is consistent with the expression seen in Panel 1.3D. Thus, the expression of this gene could be used to distinguish liver derived tissue from the toher samples in the panel, and more specifically the expression

of this gene could be used to distinguish normal liver from malignant liver tissue. Moreover, therapeutic modulation of this gene, through the use of small molecule drugs, protein therapeutics or antibodies might be of benefit in the treatment of liver cancer.

Panel 5 Islet Summary: Ag1688 Expression of the CG56155-01 gene is limited to pancreatic islets and small intestines. Please see Panel 1.3 for discussion of utility of this gene in metabolic disease.

Example 3. SNP analysis of SECX and/or NOVX clones

SeqCallingTM Technology: cDNA was derived from various human samples representing multiple tissue types, normal and diseased states, physiological states, and developmental states from different donors. Samples were obtained as whole tissue, cell lines, primary cells or tissue cultured primary cells and cell lines. Cells and cell lines may have been treated with biological or chemical agents that regulate gene expression for example, growth factors, chemokines, steroids. The cDNA thus derived was then sequenced using CuraGen's proprietary SeqCalling technology. Sequence traces were evaluated manually and edited for corrections if appropriate. cDNA sequences from all samples were assembled with themselves and with public ESTs using bioinformatics programs to generate CuraGen's human SeqCalling database of SeqCalling assemblies. Each assembly contains one or more overlapping cDNA sequences derived from one or more human samples. Fragments and ESTs were included as components for an assembly when the extent of identity with another component of the assembly was at least 95% over 50 bp. Each assembly can represent a gene and/or its variants such as splice forms and/or single nucleotide polymorphisms (SNPs) and their combinations.

Variant sequences are included in this application. A variant sequence can include a single nucleotide polymorphism (SNP). A SNP can, in some instances, be referred to as a "cSNP" to denote that the nucleotide sequence containing the SNP originates as a cDNA. A SNP can arise in several ways. For example, a SNP may be due to a substitution of one nucleotide for another at the polymorphic site. Such a substitution can be either a transition or a transversion. A SNP can also arise from a deletion of a nucleotide or an insertion of a nucleotide, relative to a reference allele. In this case, the polymorphic site is a site at which one allele bears a gap with respect to a particular nucleotide in another allele. SNPs occurring within genes may result in an alteration of the amino acid encoded by the gene at the position of the SNP. Intragenic SNPs may also be silent, however, in the case that a codon including a SNP encodes the same amino acid as a result of the redundancy of the genetic code. SNPs

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occurring outside the region of a gene, or in an intron within a gene, do not result in changes in any amino acid sequence of a protein but may result in altered regulation of the expression pattern for example, alteration in temporal expression, physiological response regulation, cell type expression regulation, intensity of expression, stability of transcribed message.

Method of novel SNP Identification: SNPs are identified by analyzing sequence assemblies using CuraGen's proprietary SNPTool algorithm. SNPTool identifies variation in assemblies with the following criteria: SNPs are not analyzed within 10 base pairs on both ends of an alignment; Window size (number of bases in a view) is 10; The allowed number of mismatches in a window is 2; Minimum SNP base quality (PHRED score) is 23; Minimum number of changes to score an SNP is 2/assembly position. SNPTool analyzes the assembly and displays SNP positions, associated individual variant sequences in the assembly, the depth of the assembly at that given position, the putative assembly allele frequency, and the SNP sequence variation. Sequence traces are then selected and brought into view for manual validation. The consensus assembly sequence is imported into CuraTools along with variant sequence changes to identify potential amino acid changes resulting from the SNP sequence variation. Comprehensive SNP data analysis is then exported into the SNPCalling database.

Method of novel SNP Confirmation: SNPs are confirmed employing a validated method know as Pyrosequencing (Pyrosequencing, Westborough, MA). Detailed protocols for Pyrosequencing can be found in: Alderborn et al. Determination of Single Nucleotide Polymorphisms by Real-time Pyrophosphate DNA Sequencing. (2000). *Genome Research.* 10, Issue 8, August. 1249-1265. In brief, Pyrosequencing is a real time primer extension process of genotyping. This protocol takes double-stranded, biotinylated PCR products from genomic DNA samples and binds them to streptavidin beads. These beads are then denatured producing single stranded bound DNA. SNPs are characterized utilizing a technique based on an indirect bioluminometric assay of pyrophosphate (PPi) that is released from each dNTP upon DNA chain elongation. Following Klenow polymerase-mediated base incorporation, PPi is released and used as a substrate, together with adenosine 5'-phosphosulfate (APS), for ATP sulfurylase, which results in the formation of ATP. Subsequently, the ATP accomplishes the conversion of luciferin to its oxi-derivative by the action of luciferase. The ensuing light output becomes proportional to the number of added bases, up to about four bases. To allow processivity of the method dNTP excess is degraded by apyrase, which is also present in the starting reaction mixture, so that only dNTPs are added to the template during the sequencing. The process has been fully automated and adapted to a 96-well format, which allows rapid screening of large SNP panels. The DNA and protein sequences for the novel single nucleotide polymorphic

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variants are reported. Variants are reported individually but any combination of all or a select subset of variants are also included. In addition, the positions of the variant bases and the variant amino acid residues are underlined.

Results

5   Variants are reported individually but any combination of all or a select subset of variants are also included as contemplated SECX and/or NOVX embodiments of the invention.

SEC1 SNP data:

SEC1 has five SNP variants, whose variant positions for its nucleotide and amino acid
10   sequences is numbered according to SEQ ID NOs:1 and 2, respectively. The nucleotide sequence of the SEC1 variant differs as shown in Table 41.

Table 41. cSNP and Coding Variants for SEC1				
NT Position of cSNP	Wild Type NT	Variant NT	Amino acid position	Amino acid change
468	T	C	130	Cys>Arg
513	T	C	145	Cys>Arg
585	A	T	169	Lys>Glu
619	G	A	180	Gly>Asp
1050	A	G	324	Thr>Ala

SEC2 SNP data:

15   SEC2 has two SNP variants, whose variant positions for its nucleotide and amino acid sequences is numbered according to SEQ ID NOs:3 and 4, respectively. The nucleotide sequence of the SEC2 variant differs as shown in Table 42.

Table 42. cSNP and Coding Variants for SEC2				
NT Position of cSNP	Wild Type NT	Variant NT	Amino acid position	Amino acid change
1894	G	A	599	Val>Met
2055	A	G	Silent	Silent

20   ### SEC4 SNP data:

SEC4 has three SNP variants, whose variant positions for its nucleotide and amino acid sequences is numbered according to SEQ ID NOs:7 and 8, respectively. The nucleotide sequence of the SEC3 variant differs as shown in Table 43.

Table 43. cSNP and Coding Variants for SEC4

NT Position of cSNP	Wild Type NT	Variant NT	Amino acid position	Amino acid change
80	T	C	11	Leu>Pro
383	T	C	112	Ile>Thr
482	A	G	145	Asn>Ser

SEC5 SNP data:

SEC5 has one SNP variant, whose variant positions for its nucleotide and amino acid sequences is numbered according to SEQ ID NOs:9 and 10, respectively. The nucleotide sequence of the SEC5 variant differs as shown in Table 44.

Table 44. cSNP and Coding Variants for SEC5

NT Position of cSNP	Wild Type NT	Variant NT	Amino acid position	Amino acid change
861	G	A	Silent	Silent

SEC7 SNP data:

SEC7 has one SNP variant, whose variant positions for its nucleotide and amino acid sequences is numbered according to SEQ ID NOs:13 and 14, respectively. The nucleotide sequence of the SEC7 variant differs as shown in Table 45.

Table 45. cSNP and Coding Variants for SEC7

NT Position of cSNP	Wild Type NT	Variant NT	Amino acid position	Amino acid change
2673	T	C	Silent	Silent

SEC10 SNP data:

SEC10 has two SNP variants, whose variant positions for its nucleotide and amino acid sequences is numbered according to SEQ ID NOs:19 and 20, respectively. The nucleotide sequence of the SEC10 variant differs as shown in Table 46.

Table 46. cSNP and Coding Variants for SEC10

NT Position of cSNP	Wild Type NT	Variant NT	Amino acid position	Amino acid change
746	G	A	234	Val>Ile
999	C	T	Silent	Silent

SEC12 SNP data:

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SEC12 has four SNP variants, whose variant positions for its nucleotide and amino acid sequences is numbered according to SEQ ID NOs:23 and 24, respectively. The nucleotide sequence of the SEC12 variant differs as shown in Table 47.

Table 47. cSNP and Coding Variants for SEC12				
NT Position of cSNP	Wild Type NT	Variant NT	Amino acid position	Amino acid change
824	G	A	Silent	Silent
849	G	T	198	Val>Phe
1215	C	A	320	Pro>Thr
1276	G	A	340	Arg>Lys

NOV1 SNP data:

NOV1 has 19 SNP variants, whose variant positions for its nucleotide and amino acid sequences is numbered according to SEQ ID NOs: 25 and 26, respectively. The nucleotide sequence of the NOV1 variant differs as shown in Table 48.

Table 48. cSNP and Coding Variants for NOV1				
NT Position of cSNP	Wild Type NT	Variant NT	Amino Acid position	Amino Acid Change
160	T	C	15	Leu>Pro
280	A	G	55	Tyr>Cys
904	A	G	263	Glu>Gly
997	T	C	294	Ile>Thr
1023	T	C	303	Cys>Arg
1041	G	A	309	Glu>Lys
1050	G	A	312	Ala>Thr
1051	C	T	312	Ala>Val
1054	A	G	313	Glu>Gly
1129	T	A	338	Leu>Gln
1207	T	C	364	Leu>Pro
1386	G	A	424	Gly>Ser
1636	A	G	507	Gln>Arg
1648	T	C	511	Val>Ala
1657	A	G	514	Glu>Gly
1680	G	A	522	Ala>Thr
1690	A	T	525	Gln>Leu
1779	A	G	555	Ile>Val
1902	G	A	596	Ala>Thr

NOV2 SNP data:

NOV2 has four SNP variants, whose variant positions for its nucleotide and amino acid sequences is numbered according to SEQ ID NOs:27 and 28, respectively. The nucleotide sequence of the NOV2 variant differs as shown in Table 49.

Table 49. cSNP and Coding Variants for NOV2				
NT Position of cSNP	Wild Type NT	Variant NT	Amino acid position	Amino acid change
590	A	G	152	Glu>Gly
1350	C	A	Silent	Silent
3252	G	A	Silent	Silent
3721	A	G	1196	Ile>Val

NOV3 SNP data:

NOV3 has six SNP variants, whose variant position for its nucleotide and amino acid sequences is numbered according to SEQ ID NOs:29 and 30, respectively. The nucleotide sequence of the NOV3 variant differs as shown in Table 50.

Table 50. cSNP and Coding Variants for NOV6				
NT Position of cSNP	Wild Type NT	Variant NT	Amino Acid position	Amino Acid Change
437	A	G	143	Asn>Ser
664	T	G	219	Phe>Val
1150	G	T	381	Ala>Ser
1210	G	T	401	Glu>Stop
1770	C	T	Silent	Silent
2011	A	G	Silent	Silent

NOV4 SNP data:

NOV4 has one SNP variant, whose variant position for its nucleotide and amino acid sequences is numbered according to SEQ ID NOs:31 and 32, respectively. The nucleotide sequence of the NOV4 variant differs as shown in Table 51.

Table 51. cSNP and Coding Variants for NOV4				
NT Position of cSNP	Wild Type NT	Variant NT	Amino Acid position	Amino Acid Change
1038	A	G	Silent	silent

NOV5 SNP data:

NOV5 has eleven SNP variants, whose variant position for its nucleotide and amino acid sequences is numbered according to SEQ ID NOs:33 and 34, respectively. The nucleotide sequence of the NOV5 variant differs as shown in Table 52.

Table 52. cSNP and Coding Variants for NOV5				
NT Position of cSNP	Wild Type NT	Variant NT	Amino Acid position	Amino Acid Change
77	T	A	Silent	Silent
86	T	C	Silent	Silent
242	C	T	Silent	Silent
367	C	T	110	Thr>Ile
421	T	C	128	Met>Thr
1301	C	T	Silent	Silent
1459	T	C	474	Leu>Pro
1475	C	T	Silent	Silent
1497	A	T	487	Thr>Ser
1526	T	C	Silent	Silent
1634	A	G	Silent	Silent

NOV6 SNP data:

NOV6 has two SNP variants, whose variant position for its nucleotide and amino acid sequences is numbered according to SEQ ID NOs:35 and 36, respectively. The nucleotide sequence of the NOV6 variants differs as shown in Table 53.

Table 53. cSNP and Coding Variants for NOV6				
NT Position of cSNP	Wild Type NT	Variant NT	Amino Acid position	Amino Acid Change
1350	A	G	442	Glu>Gly
1602	T	C	526	Val>Ala

Example 4. In-frame Cloning

NOV2 (CG56149-01)

The cDNA coding for the domain of CG56149-01 from residue 279 to 405 was targeted for "in-frame" cloning by PCR. The PCR template is based on human cDNA(s).

The following oligonucleotide primers were used to clone the target cDNA sequence:

F2 5'-GGATCC TCTGCAGCGGCTCTTTGTGTTGGAGTTGG-3' (SEQ ID NO:298)

R2 5'-CTCGAG GCCAGGTCTAGCAAGGCTTCCAAACAACATTTCC-3' (SEQ ID NO:225)

For downstream cloning purposes, the forward primer includes an in-frame BamH I restriction site and the reverse primer contains an in-frame Xho I restriction site.

Two parallel PCR reactions were set up using a total of 0.5-1.0 ng human pooled cDNAs as template for each reaction. The pool is composed of 5 micrograms of each of the following human tissue cDNAs: adrenal gland, whole brain, amygdala, cerebellum, thalamus, bone marrow, fetal brain, fetal kidney, fetal liver, fetal lung, heart, kidney, liver, lymphoma, Burkitt's Raji cell line, mammary gland, pancreas, pituitary gland, placenta, prostate, salivary gland, skeletal muscle, small Intestine, spleen, stomach, thyroid, trachea, uterus.

When the tissue of expression is known and available, the second PCR was performed using the above primers and 0.5ng-1.0 ng of one of the following human tissue cDNAs:

skeleton muscle, testis, mammary gland, adrenal gland, ovary, colon, normal cerebellum, normal adipose, normal skin, bone marrow, brain amygdala, brain hippocampus, brain substantia nigra, brain thalamus, thyroid, fetal lung, fetal liver, fetal brain, kidney, heart, spleen, uterus, pituitary gland, lymph node, salivary gland, small intestine, prostate, placenta, spinal cord, peripheral blood, trachea, stomach, pancreas, hypothalamus.

The reaction mixtures contained 2 microliters of each of the primers (original concentration: 5 pmol/ul), 1 microliter of 10mM dNTP (Clontech Laboratories, Palo Alto CA) and 1 microliter of 50xAdvantage-HF 2 polymerase (Clontech Laboratories) in 50 microliter-reaction volume. The following reaction conditions were used:

PCR condition 1:
a)   96°C   3 minutes
b)   96°C   30 seconds denaturation
c)   60°C   30 seconds, primer annealing
d)   72°C   6 minutes extension
Repeat steps b-d 15 times
e)   96°C   15 seconds denaturation
f)   60°C   30 seconds, primer annealing
g)   72°C   6 minutes extension
Repeat steps e-g 29 times
e)   72°C   10 minutes final extension

PCR condition 2:
a)   96°C   3 minutes
b)   96°C   15 seconds denaturation
c)   76°C   30 seconds, primer annealing, reducing the temperature by 1 °C per cycle
d)   72°C   4 minutes extension
Repeat steps b-d 34 times
e)   72°C   10 minutes final extension

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An amplified product was detected by agarose gel electrophoresis. The fragment was gel-purified and ligated into the pCR2.1 vector (Invitrogen, Carlsbad, CA) following the manufacturer's recommendation. Twelve clones per PCR reaction were picked and sequenced. The inserts were sequenced using vector-specific M13 Forward and M13 Reverse primers.

The insert assembly 164187747 was found to encode an open reading frame between residues 279 and 405 of the target sequence of CG56149-01. The cloned insert is 100% identical to the original amino acid sequence. The alignment with CG56149-01 is displayed in a CLUSTAL W (1.7) multiple sequence alignment below. Note that differing amino acids have a white or grey background, and deleted/inserted amino acids can be detected by a dashed line in the sequence that does not code at that position.

The cDNA coding for the FULL LENGTH of CG56149-04 from residue a to B was targeted for "in-frame" cloning by PCR. The PCR template is based on the previously identified plasmid.

The following oligonucleotide primers were used to clone the target cDNA sequence:

Primers	Sequences
F1	5'-GCGGCCGCCCACC ATGCTGAGGAGAGTCACTGTTGCT-3' (SEQ ID NO:226)
R1	5'-CTCGAG TTATTTGACTATTTTATGGTAGGGGAGAAGG-3' (SEQ ID NO:227)

For downstream cloning purposes, the forward primer includes an in-frame Not I restriction site and the reverse primer contains an in-frame Xho I restriction site.

Two PCR reactions were set up using a total of 1-5 ng of the plasmid that contains the insert for CG56149-04.

The reaction mixtures contained 2 microliters of each of the primers (original concentration: 5 pmol/ul), 1 microliter of 10mM dNTP (Clontech Laboratories, Palo Alto CA) and 1 microliter of 50xAdvantage-HF 2 polymerase (Clontech Laboratories) in 50 microliter-reaction volume. The following reaction conditions were used:

PCR condition 1:

a)	96°C	3 minutes
b)	96°C	30 seconds denaturation
c)	60°C	30 seconds, primer annealing
d)	72°C	6 minutes extension

Repeat steps b-d 15 times

e)	96°C	15 seconds denaturation
f)	60°C	30 seconds, primer annealing
g)	72°C	6 minutes extension

Repeat steps e-g 29 times

e)   72°C   10 minutes final extension

PCR condition 2:

a)   96°C   3 minutes

b)   96°C   15 seconds denaturation

c)   76°C   30 seconds, primer annealing, reducing the temperature by 1 °C per cycle

d)   72°C   4 minutes extension

Repeat steps b-d 34 times

e)   72°C   10 minutes final extension

An amplified product was detected by agarose gel electrophoresis. The fragment was gel-purified and ligated into the pCR2.1 vector (Invitrogen, Carlsbad, CA) following the manufacturer's recommendation or digested with Not I and Xho I and ligated to pFastBac1. Twelve clones per PCR reaction were picked and sequenced. The inserts were sequenced using vector-specific M13 Forward and M13 Reverse primers and the following gene-specific primers:

Primers	Sequences
SF1 SEQ ID NO:228	TCAATCTTCTCCTCAAAGCTAGAAAGA
SF2 SEQ ID NO:229	CAGAGGCTTGAAGTTTAGTTTGTCAAC
SF3 SEQ ID NO:230	GTTTGTGGTTAAATCCTTTCACTCGAATA
SF4 SEQ ID NO:231	TTTCAGCTGGAAGATGAAGATCTGG
SF5 SEQ ID NO:232	TCCTCAATTTTCCGAATCTCTTCAA
SF6 SEQ ID NO:233	TGTTAAATGCTGGTGTGTCAAATGG

SF7 SEQ ID NO:234	CTTCAACTTCACGGTCAATTGCATCT
SF8 SEQ ID NO:235	TGCTCTCTCTTCTAATTCTTCCAATTCA
SF9 SEQ ID NO:236	CAGGTAGACTTCGCCTTGTTCCTTC
SR1 SEQ ID NO:237	CTAGCTTTGAGGAGAAGATTGAGAACC
SR2 SEQ ID NO:238	CTAAACTTCAAGCCTCTGGAGCAGGAG
SR3 SEQ ID NO:239	CCACAAACTACCTCTACTGTTTCAGCTC
SR4 SEQ ID NO:240	CATCTTCCAGCTGAAAACAAGTACATAGC
SR5 SEQ ID NO:241	AATTTTTGAAGAGATTCGGAAAATTGA
SR6 SEQ ID NO:242	TGACACACCAGCATTTAACAAACTTTA
SR7 SEQ ID NO:243	GTGAATATCAACTTGCAAGGCCTTCTG
SR8	GGAAGAATTAGAAGAGAGAGCAGAAGC

SEQ ID NO:244	
SR9 SEQ ID NO:245	GAAGGAACAAGGCGAAGTCTACCTG

The insert assembly 171093681 was found to encode an open reading frame between residues 1 and 1151 of the target sequence of CG56149-04. The cloned insert is 100% identical to the original sequence. The alignment with CG56149-04 is displayed in a CLUSTAL W (1.7) multiple sequence alignment below. Note that differing amino acids have a white or grey background, and deleted/inserted amino acids can be detected by a dashed line in the sequence that does not code at that position.

NOV3 (CG56155)

The cDNA coding for the mature form of CG56155-02 from residue 20 to 638 was targeted for "in-frame" cloning by PCR. The PCR template is based on the previously identified plasmid, when available, or on human cDNA(s).

The following oligonucleotide primers were used to clone the target cDNA sequence:

Primers	Sequences
F1	5'-GGATCC GGATGTCTGACTCAACTCTATGAAAACG-3' (SEQ ID NO:246)
R1	5'-CTCGAG TGCTGGTGACTGCATCTGAGCTTTTCC-3' (SEQ ID NO:247)

For downstream cloning purposes, the forward primer includes an in-frame BamH I restriction site and the reverse primer contains an in-frame Xho I restriction site.

Two PCR reactions were set up using a total of 1-5 ng of the plasmid that contains the insert for CG56155-02.

The reaction mixtures contained 2 microliters of each of the primers (original concentration: 5 pmol/ul), 1 microliter of 10mM dNTP (Clontech Laboratories, Palo Alto CA) and 1 microliter of 50xAdvantage-HF 2 polymerase (Clontech Laboratories) in 50 microliter-reaction volume. The following reaction conditions were used:

PCR condition 1:

a)   96°C   3 minutes

b)   96°C   30 seconds denaturation

      c)   60°C   30 seconds, primer annealing

      d)   72°C   6 minutes extension

Repeat steps b-d 15 times

      e)   96°C   15 seconds denaturation

5   f)   60°C   30 seconds, primer annealing

      g)   72°C   6 minutes extension

Repeat steps e-g 29 times

      e)   72°C   10 minutes final extension

PCR condition 2:

10   a)   96°C   3 minutes

      b)   96°C   15 seconds denaturation

      c)   76°C   30 seconds, primer annealing, reducing the temperature by 1 °C per cycle

      d)   72°C   4 minutes extension

Repeat steps b-d 34 times

15   e)   72°C   10 minutes final extension

An amplified product was detected by agarose gel electrophoresis. The fragment was gel-purified and ligated into the pCR2.1 vector (Invitrogen, Carlsbad, CA) following the manufacturer's recommendation. Twelve clones per PCR reaction were picked and sequenced. The inserts were sequenced using vector-specific M13 Forward and M13 Reverse primers and the following gene-specific primers:

Primers	Sequences
SF1 **SEQ ID** **NO:248**	TTTTCTGGCATTCTTCATTTGTTACC
SF2 **SEQ ID** **NO:249**	ACTATAGATGCGCCAAACATCCTGC
SF3 **SEQ ID** **NO:250**	TCCTTACAGTCTTCTGGGAGTAAAGAA
SF4 **SEQ ID** **NO:251**	AGAAGAGGCAGTTGGGGTGATAGGT

SF5 SEQ ID NO:252	TTCAACACTGCTAACCTTAGACACATT
SR1 SEQ ID NO:253	GAAGAATGCCAGAAAAGATATCAAGATT
SR2 SEQ ID NO:254	TTGGCGCATCTATAGTGGCATTTT
SR3 SEQ ID NO:255	CCCAGAAGACTGTAAGGAAGAGAAGTGTAA
SR4 SEQ ID NO:256	CCCAACTGCCTCTTCTTTACATTCTATAC
SR5 SEQ ID NO:257	AAAAAGGTGCACCAGTAACATTCGC

The insert assembly 172884585 was found to encode an open reading frame between residues 20 and 638 of the target sequence of CG56155-02. The cloned insert is 100% identical to the original amino acid sequence. The alignment with CG56155-02 is displayed in a CLUSTAL W (1.7) multiple sequence alignment below. Note that differing amino acids have a white or grey background, and deleted/inserted amino acids can be detected by a dashed line in the sequence that does not code at that position.

NOV5 (CG56151)

The cDNA coding for the domain of CG56151-02 from residue 13 to 499 was targeted for "in-frame" cloning by PCR. The PCR template is based on the previously identified plasmid, when available, or on human cDNA(s).

The following oligonucleotide primers were used to clone the target cDNA sequence:

F3:   5'-GGATCC ACTGTCATCACTGCTGTGCTGGGTTCCTTCC-3' (SEQ ID NO:258)

R2:   5'-CTCGAG   GAATTCTGCAGCAATTTCCTCAAAAGACTTTCC-3'   (SEQ   ID
NO:259)

For downstream cloning purposes, the forward primer includes an in-frame Bam HI restriction
site and the reverse primer contains an in-frame Xho I restriction site.

5

FCA as template:

Two PCR reactions were set up using a total of 1-5 ng of the plasmid that contains the insert
for CG56151-02.

10   When the tissue of expression is known and available, the second PCR was performed using
the above primers and 0.5ng-1.0 ng of one of the following human tissue cDNAs:

skeleton muscle, testis, mammary gland, adrenal gland, ovary, colon, normal
cerebellum, normal adipose, normal skin, bone marrow, brain amygdala, brain hippocampus,
brain substantia nigra, brain thalamus, thyroid, fetal lung, fetal liver, fetal brain, kidney, heart,

15   spleen, uterus, pituitary gland, lymph node, salivary gland, small intestine, prostate, placenta,
spinal cord, peripheral blood, trachea, stomach, pancreas, hypothalamus.

The reaction mixtures contained 2 microliters of each of the primers (original concentration: 5
pmol/ul), 1 microliter of 10mM dNTP (Clontech Laboratories, Palo Alto CA) and 1 microliter

20   of 50xAdvantage-HF 2 polymerase (Clontech Laboratories) in 50 microliter-reaction volume.
The following reaction conditions were used:

    PCR condition 1:
    a)   96°C   3 minutes
    b)   96°C   30 seconds denaturation

25 c)   60°C   30 seconds, primer annealing
    d)   72°C   6 minutes extension
    Repeat steps b-d 15 times
    e)   96°C   15 seconds denaturation
    f)   60°C   30 seconds, primer annealing

30 g)   72°C   6 minutes extension
    Repeat steps e-g 29 times
    e)   72°C   10 minutes final extension
    PCR condition 2:
    a)   96°C   3 minutes

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b)   96°C   15 seconds denaturation

c)   76°C   30 seconds, reducing the temperature by 1 °C per cycle

d)   72°C   4 minutes extension

Repeat steps b-d 34 times

e)   72°C   10 minutes final extension.

An amplified product was detected by agarose gel electrophoresis. The fragment was gel-purified and ligated into the pCR2.1 vector (Invitrogen, Carlsbad, CA) following the manufacturer's recommendation.   Twelve clones per PCR reaction were picked and sequenced. The inserts were sequenced using vector-specific M13 Forward and M13 Reverse primers and the following gene-specific primers:

SF1:   CCCTGTCTGTATCCAGCTTTGCAGTT (SEQ ID NO:260)

SF2:   TATATCGGTGAAATTGCTCCAACCG (SEQ ID NO: 261)

SF3:   TGAAAAGACTCAGAGGATATGATGATG (SEQ ID NO:262)

SF4:   TTTATGCAACCATTGGAGTTGGC (SEQ ID NO:263)

SF5:   ATCTTCCTCTTTGTCAGCTTCTTTGAA (SEQ ID NO:264)

SR1:   ACCAGAGCATGGTGATTAGTTGAGC (SEQ ID NO:265)

SR2:   ATTTCACCGATATACATAGGAACCAGG (SEQ ID NO:266)

SR3:   AGCTTTGTTTTGCTTTGACTTCCTC (SEQ ID NO:267)

SR4:   ATAAACAGGTTTGCTGATACCAGCCGT (SEQ ID NO:268)

SR5:   TGGCTATCATGCTCACATAACTCATC (SEQ ID NO:269)

Results:

The insert assembly 235651305 was found to encode an open reading frame between residues 13 and 499 of the target sequence of CG56151-02. The cloned insert is 100% identical to the original sequence. The alignment with CG56151-02 is displayed in a ClustalW below. Note that differing amino acids have a white or grey background, and deleted/inserted amino acids can be detected by a dashed line in the sequence that does not code at that position.

NOV7 (CG55117)

The cDNA coding for the mature form of CG55117-04 from residue 20 to 865 was targeted for "in-frame" cloning by PCR. The PCR template is based on human cDNA(s).

The following oligonucleotide primers were used to clone the target cDNA sequence:

F1   5'-GGATCC GGAGGGCAGCCTTCATCCACAGATGCTCCTAAGG-3' (SEQ ID
NO:270)

R1   5'-CTCGAG ATGTTGTGATGGGCTTGTCATAACAGG-3' (SEQ ID NO:271)

5   For downstream cloning purposes, the forward primer includes an in-frame BamHI restriction
site and the reverse primer contains an in-frame XhoI restriction site.

Two parallel PCR reactions were set up using a total of 0.5-1.0 ng human pooled
cDNAs as template for each reaction. The pool is composed of 5 micrograms of each of the
following human tissue cDNAs: adrenal gland, whole brain, amygdala, cerebellum, thalamus,

10   bone marrow, fetal brain, fetal kidney, fetal liver, fetal lung, heart, kidney, liver, lymphoma,
Burkitt's Raji cell line, mammary gland, pancreas, pituitary gland, placenta, prostate, salivary
gland, skeletal muscle, small Intestine, spleen, stomach, thyroid, trachea, uterus.

When the tissue of expression is known and available, the second PCR was performed
using the above primers and 0.5ng-1.0 ng of one of the following human tissue cDNAs:

15   skeleton muscle, testis, mammary gland, adrenal gland, ovary, colon, normal
cerebellum, normal adipose, normal skin, bone marrow, brain amygdala, brain hippocampus,
brain substantia nigra, brain thalamus, thyroid, fetal lung, fetal liver, fetal brain, kidney, heart,
spleen, uterus, pituitary gland, lymph node, salivary gland, small intestine, prostate, placenta,
spinal cord, peripheral blood, trachea, stomach, pancreas, hypothalamus.

20   The reaction mixtures contained 2 microliters of each of the primers (original
concentration: 5 pmol/ul), 1 microliter of 10mM dNTP (Clontech Laboratories, Palo Alto CA)
and 1 microliter of 50xAdvantage-HF 2 polymerase (Clontech Laboratories) in 50 microliter-
reaction volume. The following reaction conditions were used:

PCR condition 1:

25   a)   96°C   3 minutes

b)   96°C   30 seconds denaturation

c)   60°C   30 seconds, primer annealing

d)   72°C   6 minutes extension

Repeat steps b-d 15 times

30   e)   96°C   15 seconds denaturation

f)   60°C   30 seconds, primer annealing

g)   72°C   6 minutes extension

Repeat steps e-g 29 times

e)   72°C   10 minutes final extension

.PCR condition 2:

a) 96°C 3 minutes

b) 96°C 15 seconds denaturation

c) 76°C 30 seconds, primer annealing, reducing the temperature by 1 °C per

5   cycle

d) 72°C 4 minutes extension

Repeat steps b-d 34 times

e) 72°C 10 minutes final extension

10   An amplified product was detected by agarose gel electrophoresis. The fragment was gel-purified and ligated into the pCR2.1 vector (Invitrogen, Carlsbad, CA) following the manufacturer's recommendation. Twelve clones per PCR reaction were picked and sequenced. The inserts were sequenced using vector-specific M13 Forward and M13 Reverse primers and the following gene-specific primers:

15 SF1: TTTTGTATGTGTCGTTGCTGTAACAAA (SEQ ID NO:272)

SF2: ATTCAGTGCTAGGAGGCGGAATTCTT (SEQ ID NO:273)

SF3: TGGATGCAGAACTTGACAACGTTAATA (SEQ ID NO:274)

SF4: TTTTTACTACCTGGGCTTACTGTGTGGC (SEQ ID NO:275)

SF5: TGAAGCTCACTTTTGAACAAGTTTACAG (SEQ ID NO:276)

20 SF6: CATATGATCTAGAAGCAAAAGCAAACA (SEQ ID NO:277)

SF7: GCCACCGCTCTAGATACTGCTGTT (SEQ ID NO:278)

SR1: AAATACCCCACCAGAGGCATCAGAATA (SEQ ID NO:279)

SR2: TTGATACTGTTCAGATCTGTGAACGCC (SEQ ID NO:280)

SR3: AACGTTGTCAAGTTCTGCATCCAC (SEQ ID NO:281)

25 SR4: CCACACAGTAAGCCCAGGTAGTAAAA (SEQ ID NO:282)

SR5: AATAGCTTCCCAGAGAGATAGTATTCCCA (SEQ ID NO:283)

SR6: AACTGTTTGCTTTTGCTTCTAGATCAT (SEQ ID NO:284)

SR7: CACGATGCCACTTTCTCACTGATAG (SEQ ID NO:285)

The insert assembly 188822829 was found to encode an open reading frame between

30 residues 20 and 865 of the target sequence CG55117-04. 188822829 differs from the original sequence at 2 nucleotide positions and 2 amino acid positions. It also has a 27 nuclotide/9 amino acid deletion as compared to the original sequence. The alignment with CG55117-04 is displayed in a ClustalW below. Note that differing amino acids have a white or grey

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background, and deleted/inserted amino acids can be detected by a dashed line in the sequence that does not code at that position.

NOV8 (CG56006-01)

5   The cDNA coding for the domain of CG56006-01 from residue 44 to 417 was targeted for "in-frame" cloning by PCR. The PCR template is based on the previously identified plasmid, when available, or on human cDNA(s).

The following oligonucleotide primers were used to clone the target cDNA sequence:

10   F1:   5'-AAGCTT   AGGAGTGACCAGGAGCCGCTGTACCCAGTGC-3'   (SEQ   ID   NO:286)

R1:   5'-GTCGAC GAGCTGGGTCACCATGCCGCTGGCTTCGG-3' (SEQ ID NO:287)

For downstream cloning purposes, the forward primer includes an in-frame Hind III restriction site and the reverse primer contains an in-frame Sal I restriction site.

15

FIS as template:

Two parallel PCR reactions were set up using a total of 0.5-1.0 ng human pooled cDNAs as template for each reaction. The pool is composed of 5 micrograms of each of the following human tissue cDNAs: adrenal gland, whole brain, amygdala, cerebellum, thalamus, bone

20   marrow, fetal brain, fetal kidney, fetal liver, fetal lung, heart, kidney, liver, lymphoma, Burkitt's Raji cell line, mammary gland, pancreas, pituitary gland, placenta, prostate, salivary gland, skeletal muscle, small Intestine, spleen, stomach, thyroid, trachea, uterus.

When the tissue of expression is known and available, the second PCR was performed using

25   the above primers and 0.5ng-1.0 ng of one of the following human tissue cDNAs:

skeleton muscle, testis, mammary gland, adrenal gland, ovary, colon, normal cerebellum, normal adipose, normal skin, bone marrow, brain amygdala, brain hippocampus, brain substantia nigra, brain thalamus, thyroid, fetal lung, fetal liver, fetal brain, kidney, heart, spleen, uterus, pituitary gland, lymph node, salivary gland, small intestine, prostate, placenta,

30   spinal cord, peripheral blood, trachea, stomach, pancreas, hypothalamus.

The reaction mixtures contained 2 microliters of each of the primers (original concentration: 5 pmol/ul), 1 microliter of 10mM dNTP (Clontech Laboratories, Palo Alto CA) and 1 microliter

of 50xAdvantage-HF 2 polymerase (Clontech Laboratories) in 50 microliter-reaction volume. The following reaction conditions were used:

PCR condition 1:

a)   96°C   3 minutes

b)   96°C   30 seconds denaturation

c)   60°C   30 seconds, primer annealing

d)   72°C   6 minutes extension

Repeat steps b-d 15 times

e)   96°C   15 seconds denaturation

f)   60°C   30 seconds, primer annealing

g)   72°C   6 minutes extension

Repeat steps e-g 29 times

e)   72°C   10 minutes final extension

PCR condition 2:

a)   96°C   3 minutes

b)   96°C   15 seconds denaturation

c)   76°C   30 seconds, reducing the temperature by 1 °C per cycle

d)   72°C   4 minutes extension

Repeat steps b-d 34 times

e)   72°C   10 minutes final extension.

An amplified product was detected by agarose gel electrophoresis. The fragment was gel-purified, digested with Hind III and Sal I and ligated into the Hind III and Xho I sites of the GPIV5His vector (CuraGen, New Haven, CT). Twelve clones per PCR reaction were picked and sequenced. The inserts were sequenced using vector-specific M13 Forward and M13 Reverse primers and the following gene-specific primers:

SF1:   AGCCTCCCCTCGTCCACACAGAAGAAG (SEQ ID NO:288)

SF2:   CAAAGACCATGAGCCGAGCGT (SEQ ID NO:289)

SF3:   TCTGCGCCATGTCACTGCCTCTTGTTA (SEQ ID NO:290)

SF4:   GTGATCACGGACGCAGATTGG (SEQ ID NO:291)

SF5:   CTGGATTTGCAGGGATGGGG (SEQ ID NO:292)

SR1:   CAGAGGCTGCTGGAGGTCATC (SEQ ID NO:293)

SR2:   GACAAGACGGAAGGGACGTGG (SEQ ID NO:294)

SR3:   GAAGGAGGGTGGCCGGACT (SEQ ID NO:295)

SR4:   CTCTGGCCAAGGCCCAGTC (SEQ ID NO:296)

SR5:   CCCGCTGCTGGTCAGACAC (SEQ ID NO:297)

<u>Results:</u>

The insert assemblies 235653236, 235653319, 235653473, 235653548, 235653650,

5   235653703, 235653707 and 235653711 were found to encode an open reading frame between

residues 44 and 417 of the target sequence of CG56006-01. The cloned inserts are 100%

identical to the original DNA sequence. The alignment of the amino acid sequences with

CG56006-01 is displayed in a ClustalW below. The first 33 amino acids of the ORFs from

assemblies 235653236, 235653319, 235653473, 235653548, 235653650, 235653703,

10   235653707 and 235653711 are the signal peptide encoded by the GPIV5 vector. The cloned

insert begins with the amino acids LR at positions 44 and 45 and ends with the amino acids

QL at positions 416 and 417 of the CG56006-01 peptide. The GPIV5 vector encodes the

amino acids from position 408 to the end of the assembly sequence. Note that differing amino

acids have a white or gray background while dashed lines indicate deleted or inserted amino

15   acids in the sequence.

20

OTHER EMBODIMENTS

Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims, which follow. In particular, it is

5   contemplated by the inventors that various substitutions, alterations, and modifications may be made to the invention without departing from the spirit and scope of the invention as defined by the claims. The choice of nucleic acid starting material, clone of interest, or library type is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein. Other aspects, advantages, and modifications considered to be

10   within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of treating or delaying the onset of an angiogenic-associated disorder, said method comprising administering to a subject in need thereof an antibody to the polypeptide of SEC1 in an amount sufficient to treat or prevent said angiogenic-associated disorder in said subject.

2. The method of claim 1 wherein the subject is a human.

3. The method of claim 1 wherein the angiogenic-associated disorder is selected from the group consisting of a cancer, cardiovascular disease, psoriasis, wound healing, and stroke.

4. The method of claim 1 wherein the angiogenic-associated disorder comprises endothelial cell adhesion to an extracellular matrix protein.

5. The method of claim 4 wherein the extracellular matrix protein is fibronectin or vitronectin.

6. A method for determining the presence of or predisposition to a disease associated with altered levels of SEC1 in a first mammalian subject, said method comprising

    a. Providing a protein sample from said first mammalian subject;
    b. Providing a control protein sample from a second mammalian subject known not to have or be predisposed to said disease;
    c. Measuring the amount of SEC1 polypeptide in said subject sample; and
    d. Comparing the amount of SEC1 polypeptide in said subject protein sample to the amount of SEC1 polypeptide in said control protein sample,
    wherein an alteration in the expression level of the SEC1 polypeptide in the first subject sample as compared to the control sample indicates the presence or predisposition to said disease.

7. A method for determining the presence of or predisposition to a disease associated with
    altered levels of the nucleic acid of SEC1 in a first mammalian subject, said method
    comprising

    a. Providing a nucleic acid sample from said first mammalian subject;
    b. Providing a control nucleic acid sample from a second mammalian subject
        known not to have or be predisposed to said disease;
    c. Measuring the amount of SEC1 nucleic acid in said subject sample; and
    d. Comparing the amount of SEC1 nucleic acid in said subject nucleic acid
        sample to the amount of SEC1 nucleic acid in said control nucleic acid sample,
        wherein an alteration in the expression level of the SEC1 nucleic acid in the first
subject sample as compared to the control sample indicates the presence or predisposition
to said disease.

8. A method of treating a pathological state in a mammal, the method comprising
    administering to the mammal a SEC1 polypeptide in an amount sufficient to alleviate
    the pathological state, wherein the polypeptide has an amino acid sequence at least
    95% identical to the SEC1 polypeptide, or a biologically active fragment thereof.

9. A method of treating a pathological state in a mammal, the method comprising
    administering to the mammal an antibody to a SEC1 polypeptide in an amount
    sufficient to alleviate the pathological state.

10. The method of claim 8 wherein the pathological state is selected from the group
    consisting of a cancer, cardiovascular disease, psoriasis, wound healing, and stroke.

11. The method of claim 8 wherein the pathological state comprises endothelial cell
    adhesion to an extracellular matrix protein.

12. The method of claim 11 wherein the extracellular matrix protein is fibronectin or
    vitronectin.

13. A method of treating or delaying the onset of a disorder, said method comprising
    administering to a subject in need thereof an antibody to the polypeptide of SEC1,

SEC2, SEC3, SEC4, SEC5, SEC6, SEC7, SEC8, SEC9, SEC10, SEC11, or SEC12 in an amount sufficient to treat or prevent said disorder in said subject.

14. A method of treating a pathological state in a mammal, the method comprising administering to the mammal a SEC1, SEC2, SEC3, SEC4, SEC5, SEC6, SEC7, SEC8, SEC9, SEC10, SEC11, or SEC12 polypeptide in an amount sufficient to alleviate the pathological state, wherein the polypeptide has an amino acid sequence at least 95% identical to the SEC1, SEC2, SEC3, SEC4, SEC5, SEC6, SEC7, SEC8, SEC9, SEC10, SEC11, or SEC12 polypeptide, or a biologically active fragment thereof.

15. A method for determining the presence of or predisposition to a disease associated with altered levels of SEC1, SEC2, SEC3, SEC4, SEC5, SEC6, SEC7, SEC8, SEC9, SEC10, SEC11, or SEC12 in a first mammalian subject, said method comprising

    a. Providing a sample from said first mammalian subject;
    b. Providing a control sample from a second mammalian subject known not to have or be predisposed to said disease;
    c. Measuring the amount of SEC1, SEC2, SEC3, SEC4, SEC5, SEC6, SEC7, SEC8, SEC9, SEC10, SEC11, or SEC12 in said subject sample; and
    d. Comparing the amount of SEC1, SEC2, SEC3, SEC4, SEC5, SEC6, SEC7, SEC8, SEC9, SEC10, SEC11, or SEC12 in said subject protein sample to the amount of SEC1, SEC2, SEC3, SEC4, SEC5, SEC6, SEC7, SEC8, SEC9, SEC10, SEC11, or SEC12 in said control sample,
    wherein an alteration in the expression level of the SEC1, SEC2, SEC3, SEC4, SEC5, SEC6, SEC7, SEC8, SEC9, SEC10, SEC11, or SEC12 in the first subject sample as compared to the control sample indicates the presence or predisposition to said disease.

16. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
    (a)   a mature form of an amino acid sequence selected from the group consisting of SEQ ID NOS:26, 28, 30, 32, 34, 36, 38 and 40;

(b)   a variant of a mature form of an amino acid sequence selected from the group
        consisting of SEQ ID NOS:26, 28, 30, 32, 34, 36, 38 and 40, wherein one or
        more amino acid residues in said variant differs from the amino acid sequence
        of said mature form, provided that said variant differs in no more than 15% of
        the amino acid residues from the amino acid sequence of said mature form;

(c)   an amino acid sequence selected from the group consisting SEQ ID NOS:26,
        28, 30, 32, 34, 36, 38 and 40; and

(d)   a variant of an amino acid sequence selected from the group consisting of SEQ
        ID NOS:26, 28, 30, 32, 34, 36, 38 and 40, wherein one or more amino acid
        residues in said variant differs from the amino acid sequence of said mature
        form, provided that said variant differs in no more than 15% of amino acid
        residues from said amino acid sequence.

17.   The polypeptide of claim 16, wherein said polypeptide comprises the amino acid
        sequence of a naturally-occurring allelic variant of an amino acid sequence selected
        from the group consisting SEQ ID NOS:26, 28, 30, 32, 34, 36, 38 and 40.

18.   The polypeptide of claim 16, wherein said allelic variant comprises an amino acid
        sequence that is the translation of a nucleic acid sequence differing by a single
        nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID
        NOS:25, 27, 29, 31, 33, 35, 37 and 39.

19.   The polypeptide of claim 16, wherein the amino acid sequence of said variant
        comprises a conservative amino acid substitution.

20.   An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a
        polypeptide comprising an amino acid sequence selected from the group consisting of:

        (a)   a mature form of an amino acid sequence selected from the group consisting of
                SEQ ID NOS:26, 28, 30, 32, 34, 36, 38 and 40;

        (b)   a variant of a mature form of an amino acid sequence selected from the group
                consisting of SEQ ID NOS:26, 28, 30, 32, 34, 36, 38 and 40, wherein one or
                more amino acid residues in said variant differs from the amino acid sequence
                of said mature form, provided that said variant differs in no more than 15% of
                the amino acid residues from the amino acid sequence of said mature form;

(c)   an amino acid sequence selected from the group consisting of SEQ ID NOS:26, 28, 30, 32, 34, 36, 38 and 40;

(d)   a variant of an amino acid sequence selected from the group consisting SEQ ID NOS:26, 28, 30, 32, 34, 36, 38 and 40, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence;

(e)   a nucleic acid fragment encoding at least a portion of a polypeptide comprising an amino acid sequence chosen from the group consisting of SEQ ID NOS:26, 28, 30, 32, 34, 36, 38 and 40, or a variant of said polypeptide, wherein one or more amino acid residues in said variant differs from the amino acid sequence of said mature form, provided that said variant differs in no more than 15% of amino acid residues from said amino acid sequence; and

(f)   a nucleic acid molecule comprising the complement of (a), (b), (c), (d) or (e).

21.   The nucleic acid molecule of claim 20, wherein the nucleic acid molecule comprises the nucleotide sequence of a naturally-occurring allelic nucleic acid variant.

22.   The nucleic acid molecule of claim 20, wherein the nucleic acid molecule encodes a polypeptide comprising the amino acid sequence of a naturally-occurring polypeptide variant.

23.   The nucleic acid molecule of claim 20, wherein the nucleic acid molecule differs by a single nucleotide from a nucleic acid sequence selected from the group consisting of SEQ ID NOS:25, 27, 29, 31, 33, 35, 37 and 39.

24.   The nucleic acid molecule of claim 20, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:

(a)   a nucleotide sequence selected from the group consisting of SEQ ID NOS:25, 27, 29, 31, 33, 35, 37 and 39;

(b)   a nucleotide sequence differing by one or more nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NOS:25, 27, 29, 31, 33, 35, 37 and 39, provided that no more than 20% of the nucleotides differ from said nucleotide sequence;

(c) a nucleic acid fragment of (a); and

(d) a nucleic acid fragment of (b).

25. The nucleic acid molecule of claim 20, wherein said nucleic acid molecule hybridizes under stringent conditions to a nucleotide sequence chosen from the group consisting SEQ ID NOS:25, 27, 29, 31, 33, 35, 37 and 39, or a complement of said nucleotide sequence.

26. The nucleic acid molecule of claim 20, wherein the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of:

   (a) a first nucleotide sequence comprising a coding sequence differing by one or more nucleotide sequences from a coding sequence encoding said amino acid sequence, provided that no more than 20% of the nucleotides in the coding sequence in said first nucleotide sequence differ from said coding sequence;

   (b) an isolated second polynucleotide that is a complement of the first polynucleotide; and

   (c) a nucleic acid fragment of (a) or (b).

27. A vector comprising the nucleic acid molecule of claim 26.

28. The vector of claim 27, further comprising a promoter operably-linked to said nucleic acid molecule.

29. A cell comprising the vector of claim 27.

30. An antibody that binds immunospecifically to the polypeptide of claim 16.

31. The antibody of claim 30, wherein said antibody is a monoclonal antibody.

32. The antibody of claim 30, wherein the antibody is a humanized antibody.

33. A method for determining the presence or amount of the polypeptide of claim 16 in a sample, the method comprising:

   (a) providing the sample;

(b)   contacting the sample with an antibody that binds immunospecifically to the
polypeptide; and

(c)   determining the presence or amount of antibody bound to said polypeptide,

thereby determining the presence or amount of polypeptide in said sample.

34.   A method for determining the presence or amount of the nucleic acid molecule of
claim 16 in a sample, the method comprising:

(a)   providing the sample;

(b)   contacting the sample with a probe that binds to said nucleic acid molecule; and

(c)   determining the presence or amount of the probe bound to said nucleic acid
molecule,

thereby determining the presence or amount of the nucleic acid molecule in said sample.

35.   The method of claim 34 wherein presence or amount of the nucleic acid molecule is
used as a marker for cell or tissue type.

36.   The method of claim 35 wherein the cell or tissue type is cancerous.

37.   A method of identifying an agent that binds to a polypeptide of claim 16, the method
comprising:

(a)   contacting said polypeptide with said agent; and

(b)   determining whether said agent binds to said polypeptide.

38.   The method of claim 37 wherein the agent is a cellular receptor or a downstream
effector.

39.   A method for identifying an agent that modulates the expression or activity of the
polypeptide of claim 16, the method comprising:

(a)   providing a cell expressing said polypeptide;

(b)   contacting the cell with said agent, and

(c)   determining whether the agent modulates expression or activity of said
polypeptide,

whereby an alteration in expression or activity of said peptide indicates said agent modulates
expression or activity of said polypeptide.

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40.   A method for modulating the activity of the polypeptide of claim 16, the method
        comprising contacting a cell sample expressing the polypeptide of said claim with a
        compound that binds to said polypeptide in an amount sufficient to modulate the
        activity of the polypeptide.

41.   A method of treating or preventing a NOVX-associated disorder, said method
        comprising administering to a subject in which such treatment or prevention is desired
        the polypeptide of claim 16 in an amount sufficient to treat or prevent said NOVX-
        associated disorder in said subject.

42.   The method of claim 41 wherein the disorder is selected from the group consisting of
        cardiomyopathy and atherosclerosis.

43.   The method of claim 41 wherein the disorder is related to cell signal processing and
        metabolic pathway modulation.

44.   The method of claim 41, wherein said subject is a human.

45.   A method of treating or preventing a NOVX-associated disorder, said method
        comprising administering to a subject in which such treatment or prevention is desired
        the nucleic acid of claim 5 in an amount sufficient to treat or prevent said NOVX-
        associated disorder in said subject.

46.   The method of claim 45 wherein the disorder is selected from the group consisting of
        cardiomyopathy and atherosclerosis.

47.   The method of claim 45 wherein the disorder is related to cell signal processing and
        metabolic pathway modulation.

48.   The method of claim 45, wherein said subject is a human.

49.   A method of treating or preventing a NOVX-associated disorder, said method
        comprising administering to a subject in which such treatment or prevention is desired

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the antibody of claim 15 in an amount sufficient to treat or prevent said NOVX-associated disorder in said subject.

50.   The method of claim 49 wherein the disorder is diabetes.

51.   The method of claim 49 wherein the disorder is related to cell signal processing and metabolic pathway modulation.

52.   The method of claim 49, wherein the subject is a human.

53.   A pharmaceutical composition comprising the polypeptide of claim 16 and a pharmaceutically-acceptable carrier.

54.   A pharmaceutical composition comprising the nucleic acid molecule of claim 20 and a pharmaceutically-acceptable carrier.

55.   A pharmaceutical composition comprising the antibody of claim 30 and a pharmaceutically-acceptable carrier.

56.   A kit comprising in one or more containers, the pharmaceutical composition of claim 55.

57.   A kit comprising in one or more containers, the pharmaceutical composition of claim 56.

58.   A kit comprising in one or more containers, the pharmaceutical composition of claim 55.

59.   A method for determining the presence of or predisposition to a disease associated with altered levels of the polypeptide of claim 16 in a first mammalian subject, the method comprising:

    (a)   measuring the level of expression of the polypeptide in a sample from the first mammalian subject; and

(b)   comparing the amount of said polypeptide in the sample of step (a) to the
amount of the polypeptide present in a control sample from a second
mammalian subject known not to have, or not to be predisposed to, said
disease;

wherein an alteration in the expression level of the polypeptide in the first subject as compared
to the control sample indicates the presence of or predisposition to said disease.

60. The method of claim 59 wherein the predisposition is to a cancer.

61.   A method for determining the presence of or predisposition to a disease associated with
altered levels of the nucleic acid molecule of claim 20 in a first mammalian subject, the
method comprising:

(a)   measuring the amount of the nucleic acid in a sample from the first mammalian
subject; and

(b)   comparing the amount of said nucleic acid in the sample of step (a) to the
amount of the nucleic acid present in a control sample from a second
mammalian subject known not to have or not be predisposed to, the disease;

wherein an alteration in the level of the nucleic acid in the first subject as compared to the
control sample indicates the presence of or predisposition to the disease.

62.   The method of claim 61 wherein the predisposition is to a cancer.

63.   A method of treating a pathological state in a mammal, the method comprising
administering to the mammal a polypeptide in an amount that is sufficient to alleviate
the pathological state, wherein the polypeptide is a polypeptide having an amino acid
sequence at least 95% identical to a polypeptide comprising an amino acid sequence of
at least one of SEQ ID NOS:26, 28, 30, 32, 34, 36, 38 and 40, or a biologically active
fragment thereof.

64.   A method of treating a pathological state in a mammal, the method comprising
administering to the mammal the antibody of claim 30 in an amount sufficient to
alleviate the pathological state.